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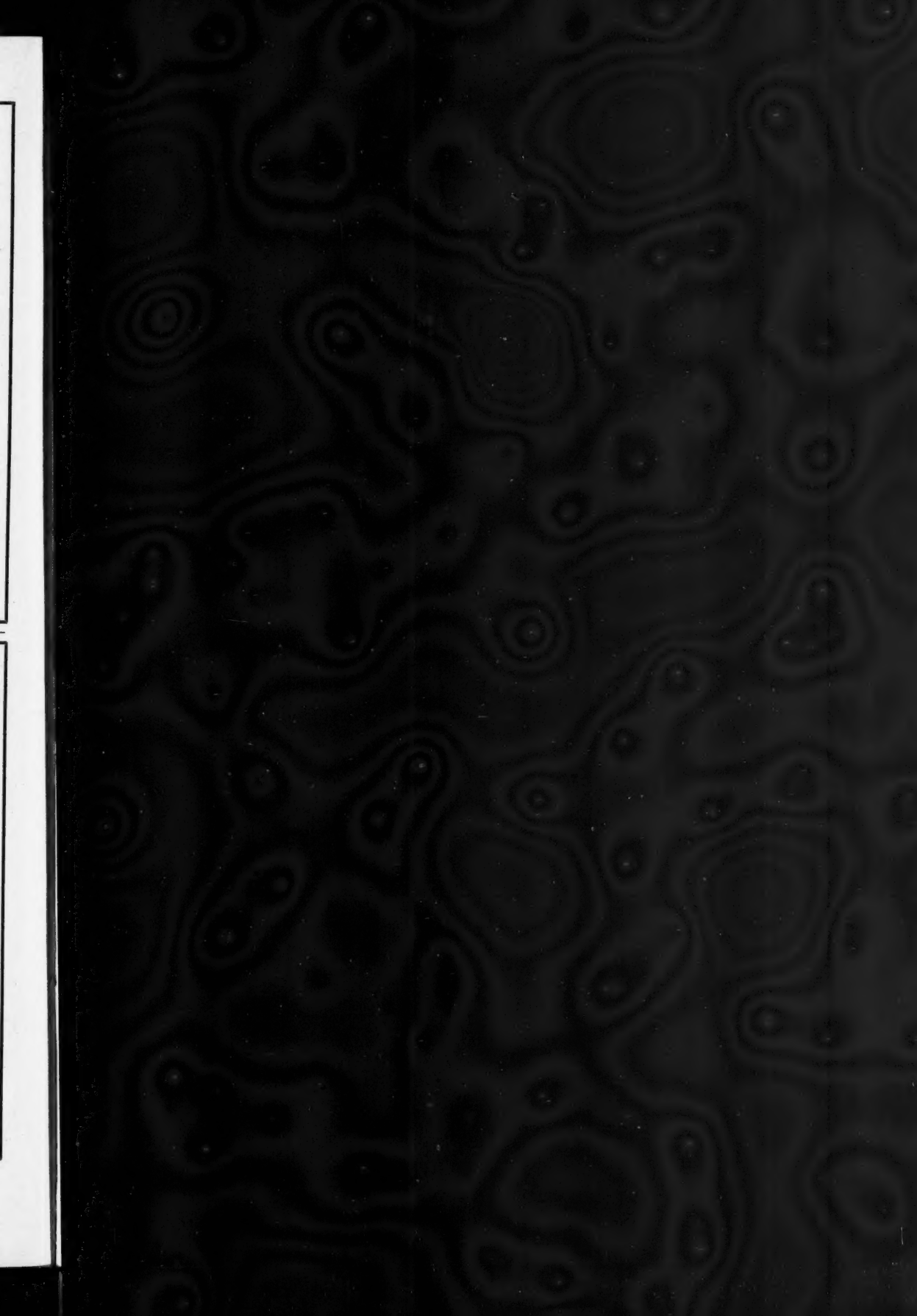

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INFLUENCE OF SOIL COLLOIDS ON AVAILABILITY OF SALTS

NEIL E. GORDON AND E. B. STARKEY

University of Maryland

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INTRODUCTION

It has long been known that clays are hydrated mixtures of iron, aluminum, silica, the alkali metals and alkali earths, and that they also contain some organic matter. When the silicates decompose, the alkali and alkali earth metals become more or less available for plant-food while a part of the iron, alumina, silica, and organic matter (humus) take on the colloidal form, and in such form are not directly available as plant food. These colloids however, play an indirect and a very important rôle in plant nutrition as the experimental work, which is to follow, will show.

Many investigators have studied adsorption on clays where the clays have been treated as a whole, but in no case up to the present have they been able to definitely tell whether adsorption occurred or whether there was a chemical reaction between the clay and the salts used. Sullivan (4) showed that changes between radicals were involved. He found many complications especially when dealing with acid and alkaline salts. Van Bemmelen (5), Ashley (1) and Moore, et al. (3) of more recent date have also done work along this line.

For some time this laboratory has been trying to throw some light on the adsorption by colloids which are known to exist in the soils, and to find the relation between the hydrogen-ion concentration and adsorption as regards these colloids. It would seem that the work has shown how some salts in certain forms become available for plant-food.

MATERIAL USED

Hydrogels of silica, alumina, and iron were prepared in the purest possible condition. The salts used in the adorption work were potassium nitrate, potassium sulfate, potassium acid phosphate, calcium nitrate, calcium sulfate, calcium acid phosphate, magnesium nitrate, magnesium sulfate, and magnesium acid phosphate. The salts were those put out by the J. T. Baker Chemical Company, under the direction of the National Research Council.

EXPERIMENTAL RESULTS

Weighed quantities of the gels were placed in glass stoppered bottles with the various solutions and shaken until equilibrium was established. The time required for the equilibrium depended upon the amount of shaking and upon the kind of gel and salt used. The solutions were analyzed before and after shaking and the adsorption obtained by difference. All the water of hydration was considered water of dilution in order that the minimum adsorption might be shown. The adsorption of each ion was calculated. In the case of calcium acid phosphate, adsorptions were obtained as shown in table 1.

In case of silica there is slight adsorption or negative adsorption. The negative adsorption would indicate that all the water which is in the gel does not act as water of dilution. Both ions are very highly adsorbed by the alumina and iron gels, and the adsorption increases with an increase of concentration.

When the silica, alumina, and iron gels had adsorbed their maximum quantity of calcium acid phosphate they were washed with hot water. The first

TABLE 1

Adsorption of calcium acid phosphate by silica, alumina, and iron gels

CONCENTRATION	Ca ADSORBED PER GRAM GEL			PO_4 ADSORBED PER GRAM GEL		
	Silica	Alumina	Iron	Silica	Alumina	Iron
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.100 *N*	−0.12	84.9	121.4	0.08	610.2	609.9
0.050 *N*	−0.12	51.4	83.3	0.04	393.4	421.6
0.025 *N*	−0.11	32.7	54.5	0.01	272.6	266.6

50 cc. of wash water was analyzed except in the case of alumina. Then 500 cc. was passed through in about 50-cc. portions and another 50-cc. portion was analyzed. This was continued during the day, and then a 50-cc. portion was allowed to stand on the gels over night. This 50 cc. was run off in the morning and analyzed in order to compare this portion with the portion obtained the day before. Table 2 gives the results for the phosphate ion.

From table 2 it is quite plain in what order the adsorbed salts are washed from the gel. The first washing gives a heavy yield of phosphate, as it takes out a large part of the salt that is mechanically held. The amount in the filtrate slowly and gradually decreases as long as there is continuous washing, but when water is allowed to stand on the gel over night, the drop is much less marked in the case of iron and alumina gels, and in the case of silica there is a noticeable increase. It took about 20 liters of washings before the phosphate was washed from the silica so that it would not give a test when water was allowed to stand on it over night. The point was not reached when the wash water of the alumina and iron gel was entirely free from phosphate.

The silica gel was washed until it did not give a trace in its washings and then the gel was decomposed and only a trace of phosphate was found. When the

washings from the alumina and iron gels began to give the small values (0.3 mgm.) given in table 2, they were decomposed and the phosphate left in the gels determined. These results are given in table 3.

TABLE 2

Results obtained by analyzing a series of washings from silica, alumina and iron gels after each had suffered a maximum adsorption of $Ca(H_2PO_4)_2$

TIME OF ANALYSIS	PO_4 FOUND IN 50 CC. OF WASH WATER		
	Silica gel	Alumina gel	Iron gel
	mgm.	*mgm.*	*mgm.*
First 50 cc.	72.9		39.9
After 500 cc.	0.5	4.0	7.8
After 1000 cc.	0.4	2.6	3.7
After 1500 cc.	0.3	2.3	2.4
After first night	5.9	2.6	2.1
After 3000 cc.	0.0	2.0	1.8
After second night	4.0	1.8	1.8
After 5000 cc.	0.0	0.3	0.2
After third night	Trace	0.3	0.3

TABLE 3

Results obtained by analyzing alumina and iron gels for PO_4 radical before and after washings

TIME OF ANALYSIS	PO_4 IN GEL	
	Alumina gel	Iron gel
	gm.	*gm.*
Before washing	0.3142	0.5728
After washing	0.2308	0.2688

TABLE 4

Results which show how adsorption varies with the hydrogen-ion concentration

pH VALUE	ADSORPTION PER GRAM SILICA GEL		pH VALUE	ADSORPTION PER GRAM IRON GEL	
	Ca	PO_4		Ca	PO_4
	mgm.	*mgm.*		*mgm.*	*mgm.*
4.243	0.45	−0.64	5.156	109.5	554.8
4.193	0.41	−0.75	5.156	104.5	536.8
4.193	0.36	−1.00	5.038	101.9	536.8
4.142	0.18	−1.56	5.021	101.9	581.7
2.463	−0.89	−2.62	4.446	56.4	587.7

The alumina gel contained about two-thirds of the original phosphate radical, while the iron gel contained about one-half.

One-twentieth normal solutions were made of different salts and their hydrogen-ion concentration was varied by the addition of sodium hydroxide or hydrochloric acid as the case might require. The adsorption in the case of calcium acid phosphate is given in table 4.

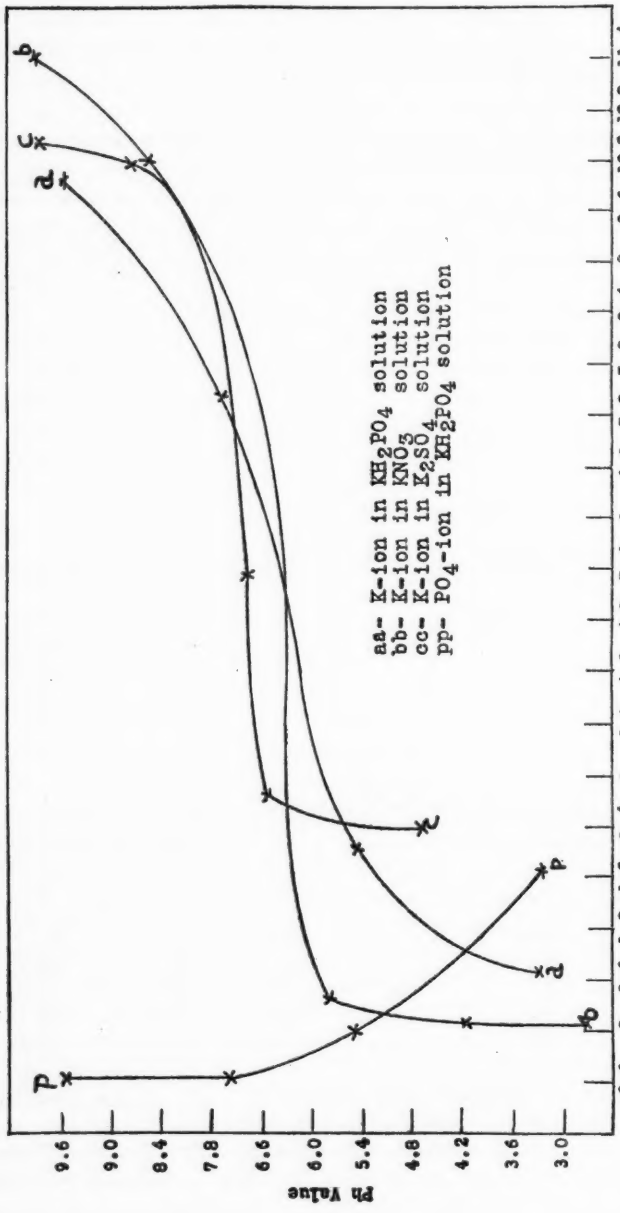
aa- K-ion in KH_2PO_4 solution
bb- K-ion in KNO_3 solution
cc- K-ion in K_2SO_4 solution
pp- PO_4-ion in KH_2PO_4 solution
Mgm. Adsorbed per gm. Gel.
Ph Value
-0.6 0 0.6 1.2 1.8 2.4 3 3.6 4.2 4.8 5.4 6 6.6 7.2 7.8 8.4 9 9.6 10.2 10.8 11.4
9.6 9.0 8.4 7.8 6.6 6.0 5.4 4.8 4.2 3.6 3.0
a a
b b
c c
p p

FIG. 1. RELATION OF HYDROGEN-ION CONCENTRATION TO ADSORPTION OF POTASSIUM BY SILICA GELS

Table 4 shows how the amount of calcium adsorbed in each gel decreases with an increase of hydrogen-ion concentration. The phosphate radical follows the same order in the silica gel, but in the iron gel the order seems to be somewhat broken. The change of adsorption is not so marked in any case because of the small change in hydrogen-ion concentration. The hydrogen-ion concentration had to be kept within such limits that the calcium acid phosphate would not be precipitated on the one hand and that the iron would not dissolve on the other hand.

How the adsorption of potassium varies with a change of hydrogen-ion concentration when silica gel is treated with various potassium salts, is shown in table 5.

Potassium in potassium salts follow the same order as calcium in calcium salts but table 5 shows that in the case of silica gel potassium is much more adsorbed, and further shows a greater change of adsorption with a change of hyrogen-ion concentration. A graph, figure 1, of the results of table 5 brings

TABLE 5

Variation of adsorption of potassium in relation to hydrogen-ion concentration when solutions of different potassium salts are used with silica gel

ADSORPTION OF KH_2PO_4		ADSORPTION OF KNO_3		ADSORPTION OF K_2SO_4	
pH value	Amount per gram of gel	pH value	Amount per gram of gel	pH value	Amount per gram of gel
	mgm.		*mgm.*		*mgm.*
9.501	9.63	9.873	11.2	9.890	10.06
7.692	6.56	8.554	9.8	8.706	9.93
6.086	1.74	6.830	2.6	7.337	4.70
3.888	−0.68	6.390	−1.1	7.117	1.91
		3.360	−1.3	5.275	1.16

out the relation between hydrogen-ion concentration and adsorption. It is noted by the graph that when the hydrogen-ion concentration is about 7, a small change in hydrogen-ion concentration is accompanied by a large change in adsorption.

DISCUSSION OF RESULTS

The results in table 1 would indicate that the adsorption of soils is influenced more by iron and alumina colloids than by silica colloids. The alumina and iron colloids would have about equal influence. The concentration of the soil solutions should have some influence on the quantity of salt adsorbed by the soil colloids.

Table 2 gives one some idea of how salts may become available after they have once been adsorbed by the colloids of the soil. The adsorbed quantity, which would probably be the amount left after the second or third washing is seen to be released in a very gradual fashion. This indicates that soil colloids play a rôle in keeping salts from being washed quickly from the soil. When

the water is allowed to stand on the gel over night, the salt is given time to partition itself to a greater extent between the gel and the water. In the adsorption experiments it was found that the gels reached an equilibrium very slowly, and in a like manner they give back their salts very slowly, but probably fast enough for the use of the salt for the plant. This fact is especially true of the iron and alumina gels. Whether all the salt adsorbed by the alumina and iron gels is available for plant food is questionable.

The results as given in table 4 and the accompanying graph point to the fact that the adsorption by soil colloids is influenced to some extent, at least, by the acidity of the soils. Potassium and calcium in certain forms, at least, are less available as the hyrogen-ion decreases. In the graph, figure 1, it will be noted that a small change in the hydrogen-ion concentration around the value 7 is followed by a big change in adsorption of potassium by silica gel.

The work seems to be in keeping with some investigational work on soils. The work of A. G. McCall and A. M. Smith (2) is an illustration. They added sulfur to different greensand marls, and varied conditions so that varying amounts of sulfur were oxidized over to the sulfur acids. Where the greatest amount of sulfur was oxidized the largest quantity of water soluble potassium was produced. According to table 4, when the hydrogen-ion concentration is increased by the oxidation of the sulfur, the potassium should become less adsorbed or more available. This is what was found in the reference given.

The probable facts are that the outer surfaces of these silicate grains have undergone decomposition to the extent that the iron, alumina, and silica in this outer layer have taken on the colloidal form. Each sand grain has become, as it were, encased in a thin colloidal layer and the potassium is more or less adsorbed by this colloid. As soon as the sulfur begins to be oxidized, and hence the hydrogen-ion concentration increased, the potassium, according to table 4, should be less adsorbed by this encasing colloid or become more available. This is what McCall and Smith found. Furthermore, they found that the fineness of the sand increased the availability of the potassium. Since the fineness of the sand means more surface, and since adsorption is a surface phenomena this increased availability of the potassium would be expected when the hydrogen-ion concentration is increased by the addition of sulfur.

SUMMARY

1. Silica gel shows small adsorption for salts like calcium acid phosphate, while the alumina and iron gels show a large power of adsorption.

2. Within the limits of the concentrations used, the adsorption increased with the concentration of the salt solution.

3. The colloids gave up their adsorbed salts gradually.

4. One of the factors affecting the solubility and therefore the availability of some ions like potassium and calcium in certain forms is the hydrogen-ion concentration.

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VARIATION OF NITRATE NITROGEN AND pH VALUES OF SOILS FROM THE NITROGEN AVAILABILITY PLOTS[1]

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The nitrogen availability plots at this station have been under observation since 1908 and for this reason they seemed to offer an excellent opportunity for a study of the variations in pH values and nitrate nitrogen in the soil during and immediately following the growing season.

It was therefore determined to take samples at intervals of about two weeks through the summer and continuing into the fall as long as samples could be taken to good advantage.

The crop rotation which has been used and the fertilizer treatment have been described in earlier publications (2, 3) and on that account only brief reference need be made here to these points. The soil is a loam, but inclines to the gravelly phase.

The rotation is a general farm crops rotation—corn, oats, wheat and timothy (two years) without legumes, since these could not be introduced where the availability of nitrogenous fertilizers is being studied. The series of plots marked *A* receive no lime; those marked *B* received ground limestone at the rate of one ton per acre in 1908 when the work was started, and since that time, at intervals of five years, have received two tons per acre. The last application was made in the spring of 1918.

The fertilizer treatment for the plots included has been as follows:

PLOT NUMBER	FERTILIZER TREATMENT
7A, 7B	Nothing
9A, 9B	Minerals,[1] 16 pounds $NaNO_3$
11A, 11B	Minerals, $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$
13A, 13B	Minerals, dried blood equivalent to 16 pounds $NaNO_3$
17A, 17B	Minerals, 200 pounds wheat or rye straw
18A, 18B	Minerals, 1600 pounds cow manure and 16 pounds $NaNO_3$
19A, 19B	Minerals, only

[1] Minerals = 32 pounds acid phosphate and 16 pounds muriate of potash.

Fertilizers and manures are applied annually, near the time of seeding the crop, except in the case of fall-sown crops, when only one-fourth of the mineral

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nitrogenous fertilizer is applied at the time of seeding and the remainder as a top dressing the following spring. Also in the case of the hay crop the mineral fertilizers are applied in the fall following the first season's cutting.

It should be explained here that the wheat which was seeded in the fall of 1919 was badly injured by the cold weather of 1919–1920 and on this account it became necessary to plow this under and reseed to barley in the spring of 1920. The barley was seeded on May 3. Nearly a month later—May 29—the first samples of soil were collected. Five or more borings, to the depth of $6\frac{2}{3}$ inches, were taken from each plot, and these were combined to make a composite sample. These samples were taken to the laboratory where they were air-dried and passed through a 2 mm. sieve (without grinding) to remove gravel and course organic matter. Following the barley the land was plowed during the latter half of August and fitted for seeding timothy. Manure and fertilizers were applied in accordance with the plan already outlined, the former being spread broadcast before the land was plowed and the latter after the land was prepared, September 3 and 4. The timothy was seeded September 7.

The hydrogen-ion determinations were made by the double tube color method described by Gillespie (1) and the nitrate determinations by the colorimetric method of Schreiner and Failyer (5).

The results of the hydrogen-ion determinations (pH values) are shown in table 1. It is at once apparent that there has been little change in the pH values throughout the period, the averages for the various samplings being almost identical, with the exception of the samples taken May 29th.

There is on the other hand a distinct difference between samples from the limed and the unlimed plots, the latter showing distinctly lower pH values, the lowest season-average for any plot being 4.7 (11A), and the highest 6.1 (9A and 18A).

It will be recalled that 11A is the plot which has received annual applications of ammonium sulfate equivalent to 320 pounds of nitrate of soda per acre. This plot is now so acid that it practically inhibits growth of such crops as corn, wheat, and timothy. Instead there appears a volunteer crop of sour grass, and later in the season a heavy growth of crab grass.

From the results of this work it would appear that a pH value in the neighborhood of 4.5 indicates a degree of acidity which practically prohibits the growth of the ordinary farm crops. This view is strengthened by results secured where the soil was made very acid by heavy applications of sulfur (4). In this case the growth of soy beans was very much reduced where the pH value was 4.5 or less.

With the exception of 11B (the limed ammonium sulfate plot) the limed plots show a pH value varying but little from 7.0. However the samples taken on May 29th and November 25th appear to be slightly lower in pH value than those taken at the intervening dates.

The samples from 11B are consistently lower in pH value than those from the other plots, although the soil of this plot is not sufficiently acid to interfere with normal crop growth.

It is of interest to compare pH values of samples from these plots with the lime requirement determination by the Veitch method. These determinations were made for all of the plots, but at less frequent intervals than the pH readings. It does not seem wise to attempt a direct comparison with the pH values, but it is of interest in connection with these values to note that the average lime requirement for plot 11A is more than four times as much as for 9A.

TABLE 1

Hydrogen-ion concentration, expressed as pH values, of soils from the nitrogen availability fertilizer plots. May 29 to November 25, 1920

PLOT NUMBER	MAY 29	JUNE 14	JUNE 28	JULY 12	JULY 26	AUGUST 9	AUGUST 24	SEPTEMBER 13	SEPTEMBER 27	OCTOBER 11	OCTOBER 25	NOVEMBER 11	NOVEMBER 25	AVERAGE
Unlimed section														
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
7A*	5.8	5.8	6.0	5.8	6.0	6.0	6.0	5.8	6.0	6.2	6.2	6.1	6.0	5.97
9A	6.0	6.4	6.3	6.1	6.0	6.0	5.9	6.0	6.0	6.2	6.1	6.0	6.0	6.07
11A	4.6	4.5	4.6	4.7	4.6	4.8	4.9	4.9	4.5	4.7	4.9	4.7	4.7	4.70
13A	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.9	5.9	5.9	5.8	6.0	5.9	5.79
17A	5.8	6.0	6.2	6.0	5.8	5.8	5.8	5.8	5.9	5.8	5.8	5.9	6.0	5.89
18A	5.9	6.2	6.2	6.2	6.0	6.1	6.3	6.2	5.9	6.0	6.0	6.0	6.0	6.07
19A	5.7	5.4	5.4	5.9	5.7	5.7	5.9	6.0	5.7	5.8	5.9	5.8	5.9	5.75
Average	5.61	5.70	5.73	5.76	5.63	5.70	5.75	5.80	5.65	5.73	5.75	5.73	5.70	5.71
Limed section														
7B	6.9	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.1	7.1	7.0	7.0	7.0	7.01
9B	7.1	7.2	7.1	7.2	7.1	7.2	7.2	7.1	7.2	7.0	7.0	7.1	7.1	7.02
11B	6.4	6.5	6.4	6.8	6.5	6.7	6.7	6.8	6.6	6.6	6.6	6.4	6.4	6.56
13B	6.9	6.9	6.9	6.9	7.0	7.0	7.1	7.1	7.1	7.0	7.0	6.8	6.9	6.96
17B	6.9	7.2	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2	7.0	6.9	6.8	7.06
18B	6.9	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.1	7.0	6.9	7.08
19B	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.1	7.1	6.9	7.13
Average	6.89	7.00	6.96	7.03	7.00	7.04	7.06	7.07	7.08	7.04	6.97	6.90	6.86	6.99

*Omitted from average on account of the difficulty in getting a clear solution for readings.

The lime requirement appears to vary some with the date of sampling, but this variation may be apparent rather than real, since it is difficult to get close agreement of results by this method.

Of the limed plots, 9A shows a lower average requirement than any of those that received fertilizer. This is in comformity with the generally accepted opinion that the continuous use of nitrate of soda tends toward an alkaline condition of the soil. In this connection it may be pointed out that 9A shows a higher pH value than any of the other unlimed plots except 18A, and this plot also receives nitrate of soda in addition to the manure.

Of the limed plots only 11B showed a lime requirement by the Veitch method, and this is so low as to be practically negligible. The lime requirement determinations are shown in table 2.

TABLE 2

Lime requirement (pounds CaO per 2,000,000 pounds soil) by the Veitch method. 1920

DATE OF SAMPLING	7A	9A	11A	13A	17A	18A	19A	AVERAGE
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
May 29	400	600	3200	1400	1200	800	1200	1257
July 12	600	600	2000	800	800	800	1200	971
August 24	400	600	1600	1200	400	800	1200	886
October 11	200	200	2000	600	1000	1000	1200	886
November 25	600	600	1800	600	1000	1000	800	771
Average	360	520	2120	920	760	880	1120	954

INFLUENCE OF SOIL REACTION ON CROP YIELDS

In connection with the pH values and lime requirement data it is of interest to study the yields of barley and of timothy hay obtained from these plots for the seasons of 1920 and 1921. The weights for the different plots are shown in table 3 which appears under the discussion of nitrates. From these figures it will be noted that the limed plots gave the largest yield in nearly every case. The low yields from 7A and 7B are due in part to starvation, these plots having received no fertilizer during the entire period. The wide difference between the yields on 11A and 11B is of especial interest in connection with the pH values and lime requirement determinations for these plots, the yield of barley on the latter being eight times, and of timothy hay nearly twenty-five times that on the former. In connection with the timothy hay of 1921 it should be pointed out, that 11A yielded a rather heavy second growth crop, 660 pounds, which was practically all crab grass. On the other hand there was not enough second crop growth on 11B to justify cutting.

The increase in yield from 17B over 17A is due in part to volunteer clover which has been gradually creeping in on this plot. The tendency of the clover to come in is due to the fact that this plot receives its nitrogen in the form of chopped-up rye straw, a slowly available source, and the lime and minerals which are applied encourage the growth of clover to the exclusion of the timothy which does not get enough available nitrogen. A considerable amount of volunteer clover has also appeared on 19B and this accounts in part for the increase in yield over 19A. These two cases only serve to further emphasize the importance of keeping the soil reaction near the neutral point where clover is to be grown.

NITRATE NITROGEN IN THE SOILS

Nitrate determinations for the seven limed and seven unlimed plots, for the period May 29 to November 25, 1920, are shown in table 3.

With comparatively few exceptions the unlimed plots show a higher nitrate content than the limed plots. This is clearly brought out by the averages for

TABLE 3

Nitrate nitrogen expressed as parts per million of nitrogen in soil samples from unlimed and limed plots collected at intervals of two weeks from May 29 to November 25, 1920

PLOT NUMBER	MAY 29	JUNE 14	JUNE 28	JULY 12	JULY 26	AUGUST 9	AUGUST 24	SEPTEMBER 13	SEPTEMBER 27	OCTOBER 11	OCTOBER 25	NOVEMBER 8	NOVEMBER 25	AVERAGE
						Unlimed section								
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
7A	2.52	1.26	4.12	2.12	2.10	0.72	1.46	5.13	5.73	4.00	8.79	8.79	1.10	3.68
9A	5.65	0.88	2.14	1.61	1.20	1.08	4.00	6.91	11.46	6.87	4.24	1.80	0.80	3.74
11A	8.27	13.18	20.78	16.46	0.66	0.50	2.26	4.00	7.99	3.49	7.99	7.99	1.72	7.33
13A	14.39	1.76	1.44	2.00	0.80	0.72	2.52	3.32	7.03	4.00	2.50	1.53	1.34	3.33
17A	2.60	0.72	1.30	1.06	0.80	0.61	1.04	1.70	2.40	2.66	3.66	2.28	1.00	1.67
18A	13.79	2.80	4.19	3.46	3.06	4.60	2.92	15.98	21.98	9.99	14.59	13.26	1.80	8.63
19A	3.25	1.16	1.34	1.90	0.80	0.53	0.81	2.00	2.54	2.00	2.20	3.04	0.64	1.71
Average	7.21	3.11	5.04	4.09	1.35	1.25	2.14	5.58	8.45	4.72	6.28	5.53	1.20	4.30
						Limed section								
7B	3.86	1.26	1.40	2.00	1.60	0.69	1.33	6.63	13.83	2.00	4.00	4.63	0.80	3.39
9B	5.32	0.46	1.60	1.75	3.20	1.00	1.90	7.43	7.99	2.00	3.06	1.06	0.72	2.88
11B	7.99	1.00	1.54	2.00	2.00	1.36	1.04	4.80	4.00	3.66	3.20	3.60	0.80	2.85
13B	5.51	0.92	1.50	2.12	2.00	0.90	1.72	3.52	5.27	3.66	5.00	6.39	1.60	3.09
17B	5.79	3.20	2.60	4.00	3.00	1.44	1.90	4.63	6.63	2.00	1.80	1.80	1.00	3.06
18B	9.31	0.88	3.20	4.00	3.46	3.32	4.52	15.98	22.37	6.33	1.91	2.00	1.60	6.07
19B	3.39	0.94	2.14	2.04	2.26	1.76	2.32	5.31	7.19	1.28	2.60	0.88	1.60	2.59
Average	5.88	1.24	2.00	2.56	2.50	1.50	2.10	6.90	9.61	2.99	3.08	2.91	1.16	3.42

the two sections, the general average for the former being 4.30 parts of nitrogen per million and for the latter 3.42 parts per million.

The larger growth of barley on the limed plots during the early part of the season might easily account for this difference. On the other hand there is some doubt as to whether this explanation would hold after the barley was full ripe and had been harvested, that is, after about July 26.

During the early part of the season the barley made very little growth on 11A and this was reflected in the higher content of nitrates in the soil up to July 12. The low nitrate content of this soil for July 26 and August 9 appears to be accompanied by a low content on nearly all the other plots. Since the grain

was full ripe on July 26 and was harvested between that date and the end of the month, it could hardly be attributed to the withdrawal of nitrogen by the growing crop.

The rainfall during the months of July and August was heavy—over 14 inches for the two months—and it is entirely possible that this may have kept the nitrates washed below the depth to which soil samples were taken. During September and October when the amount of nitrate nitrogen was greater, the rainfall averaged only a little over two inches for the two months. It must not be forgotten, however, that the application of the fertilizer early in September had something to do with the higher nitrate content at this time. Likewise the high average content of nitrates for May 29 is undoubtedly a reflection of the nitrogenous fertilizers which were applied to certain of these plots (no. 9, 11, 13, 17 and 18) on April 9.

The average nitrate content of the soil from plot 11A was 7.33 parts per million which is next to the highest average, the highest being 8.63 from plot 18A, which receives annually a heavy application of manure in addition to the nitrate of soda.

From November 8 to 25 there was a rather abrupt drop in the amount of nitrates for nearly all plots. As the first freezing weather occurred about November 12 to 14, the lower temperatures from this time on would no doubt account for the slowing down of nitrification.

The low average for 17A may be attributed to the fact that this plot receives rye straw which furnishes a comparatively small amount of nitrogen in a slowly available form. Plot 19A receives no nitrogen and here also the average is low.

There is less variation in the amount of nitrate nitrogen in the soils from the limed than from the unlimed section, the average being not quite three parts per million if 18B be excepted.

The use of lime and mineral fertilizers on 17B and 19B has encouraged the growth of volunteer clover on these plots, and this undoubtedly accounts, in part, for the higher production of nitrates on these plots than on the corresponding unlimed plots.

The highest average nitrate content for both sections occurred on September 27 when the plots of the unlimed section gave an average of 8.45 parts of nitrogen per million and the plots of the limed section 9.61 parts per million. Two weeks later the average of the plots for the limed section was less than one-third what it was on September 27. The reason for the high content of nitrates for the two samplings in September has already been referred to.

NITRATE FORMATION AND CROP YIELD

As already pointed out plot 11A shows a higher season average of nitrates found in the soil than 11B, and yet the yield of barley (grain) on the former for 1920 was approximately $\frac{1}{15}$, and the timothy hay in 1921, only $\frac{1}{8}$ of that on the latter.

It is thus quite clear that growth was hindered by something else than a lack of available nitrogen, and that an acid soil does not necessarily inhibit nitrification. In an earlier publication (4) attention has been called to this latter fact.

The yields for the two crops are shown in table 4.

It will be noted that the yield of timothy hay on plots 9A and 9B, 13A and 13B, and 18A and 18B do not differ greatly, although 18A and 18B receive about four times as much nitrogen every year as the others. From this it is evident that there must be a very great loss of nitrogen from these two plots.

This excessive application of nitrogen tends to produce a hay or grain crop with a weak straw and in the case of grain crops, an over abundance of leaf and stem growth as compared with grain and consequently there is a considerable loss on account of lodging.

TABLE 4

Yield of barley (grain) and timothy hay on unlimed and limed plots 1920 and 1921

PLOT NUMBER	BARLEY, 1920		TIMOTHY HAY, 1921	
	A (Unlimed)	B (Limed)	A (Unlimed)	B (Limed)
	lbs.	*lbs.*	*lbs.*	*lbs.*
7	81	568	151	650
9	1308	1244	2880	2960
11	56	1360	450	3620
13	1112	1240	2700	3000
17	864	1340	1580	2120
18	1888	2040	2760	3440
19	604	960	870	1650

During the spring and early summer of 1921 there was a very heavy growth of chick weed on plots 18A and 18B, and this interfered very seriously with the growth of the timothy. It would appear that the abundant supply of nitrates is especially favorable to the growth of this weed. It is thus evident that an over-supply of nitrates may have a distinctly detrimental effect.

The low yields on plots 7A and 7B furnish a good illustration of how the yields go down on land that is continuously unmanured and unfertilized. It would appear that the lime continues to aid in unlocking slowly available nitrogen and mineral plant food and thus the yield on the limed plot is kept considerably above that on the corresponding unlimed plot. However, the average amount of nitrates is very nearly the same on the two plots.

It is only a matter of time when even the limed plot will fail to give a crop worth harvesting if the same treatment is continued.

In the case of 17B the lime has apparently aided in breaking down the slowly available organic matter and this has resulted in a larger supply of nitrates for the crop, but as previously pointed out this effect may be apparent rather than real, and the larger crop yield and higher nitrate concentration may be due to

the volunteer clover which has been creeping in from year to year. On account of a deficiency of lime there is no tendency for volunteer clover to appear on the corresponding unlimed plot, 17A.

SUMMARY

Hydrogen-ion determinations on sampes of soil collected at intervals of two weeks, over a period of nearly 5 months, showed only slight variations in pH values for a given fertilizer treatment.

On the unlimed section the pH values varied considerably according to the fertilizer treatment given the different plots; the variation was much less, for the same treatment, on the limed section. The pH values were quite consistently lower on the unlimed than on the limed plots. The lowest pH values for both sections were found on the ammonium sulfate plot, but the soil from the unlimed plot was much more acid than that from the limed plot.

The plot having the lowest pH value throughout the period (11A) shows the highest lime requirement by the Veitch method.

The unlimed nitrate of soda plot (9A) shows a slightly higher pH value than most of the other plots in this section and a lower lime requirement (excepting the unfertilized plot, 7A).

Those plots which gave a pH value of about 7 showed no lime requirement by the Veitch method.

From the above it is concluded that for normal soils a determination of the pH values may be of considerable assistance in arriving at the amount of lime required for general farm crops.

The bi-weekly nitrate determinations showed considerable variation as the season progressed, the lowest period being about the last of July and through August, just after the crop had been removed.

The high results obtained at the two samplings in September are the result, in part at least, of the fertilizers which were applied on September 3 and 4.

The averages for the unlimed plots are, with some exceptions, higher than the averages for the limed plots.

A high content of nitrates does not necessarily mean a good crop yield, there may be other inhibiting factors.

Nitrates may be found in considerable quantities in a soil that is so acid as to practically inhibit growth of ordinary farm crops. Such acidity does not seem to inhibit the growth of crab grass.

There appears to be a slowing down of nitrification with the approach of cold weather (freezing temperature).

A large excess of available nitrates is likely to cause lodging of grain and hay, with a consequent loss in yield.

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FIG. 1. THE BARLEY ON PLOT 11A, WITHOUT LIME, WAS A FAILURE

FIG. 2. PLOT 11B WITH THE SAME FERTILIZER TREATMENT PLUS LIME PRODUCED A GOOD CROP OF BARLEY

A POSSIBLE CORRELATION BETWEEN THE FERTILITY OF RICE SOILS AND THEIR TITRATION CURVES

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In Java, as throughout the East, rice-culture plays a very important rôle, because rice is the essential food of the natives. However, the population has grown so rapidly that rice cultivation has not been able to keep pace with it. In order to increase the rice crop, several steps have been taken, such as cultivation by machinery at Selatdjaran, (5) colonization of new land, and improvement of old sawahs.[1] New ways of meeting the situation are well worth trying, and these efforts were made with that end in view.

The rice crop in Java varies from about 100 to 5 pikkols paddi per bouw, with an average of 25 pikkols per bouw for all of Java (that is, 2000 kgm. paddi, or 1000 kgm. rice per hektare). Soils giving less than 25 pikkols per bouw are considered bad.

The causes of bad crops are either external as in the case of too much or too little water, or when the soils are improperly worked, or they are internal, as in the case of bad or unhealthy soil or those becoming easily infected. If one could ascertain the reasons for soils being bad, one could look for a possibility of improving them.

It is not a simple condition with which we are dealing, but a complex of physiological and pathological conditions. Why does a plant become diseased? In most cases it is because the plant lacks strength in one way or another. The basis of our work will be the fact that bad soils cause a weakness of the plant.

Many authors (3, 7) have found that it is possible to classify soils into natural groups according to the hydrogen-ion concentration, expressed in terms of pH values. Since the influence of the hydrogen-ion concentration upon the growth of plants is well known, the possibility suggested itself that some valuable information could be obtained in an attempt to solve the question of how rice soils become bad through an investigation of the hydrogen-ion concentration.

Several soils of different types were used for this work. Ten grams of soil and 50 gm. of water were shaken and filtered. The pH value was determined colorimetrically according to the method of Gillespie (2). The results are given

[1] A sawah is a rice field under water.

in table 1 which does not show any correlation between the pH values and the various soils, except in two instances. The four most acid soils are bad and the five most alkaline are good. Except for those two groups there is no cor-

TABLE 1

The correlation between the crop productivity of rice soils and their pH values

SOIL NUMBER	LOCATION AND TYPE	PRODUCTIVITY	REACTION
	Madoera, Sapoeloe	Good	8.2
5851	Preanger, Djampangkoelon	Good	8.1
5085	Semarang, Demak III	Good	8.0
	Semarang, Demak Rimboelor	Good	7.8
	Soerabaja, Lebo	Good	7.8
1961	Soerabaja, Modjokasri	Rather bad	7.8
	Madoera, Gedoengan	Good	7.6
	Soerabaja, Kedamean	Bad	7.6
	Soerabaja, Kedamean	Bad	7.6
6747	Cheribon, Kantji	Bad	7.5
5087	Semarang, Dempet	Bad	7.5
5856	Preanger, Djampangkoelon	Bad	7.4
	Soerabaja, Lebo	Good	7.4
1957	Soerabaja, Madjoeagong	Good	7.3
1958	Soerabaja, Djabang Pandan	Good	7.3
	Madoera, Manding	Good	7.3
6204	Preanger, Tjiawi	Bad	7.3
1959	Soerabaja, Djombang	Good	7.2
6751	Cheribon, Nieuw Tersana	Good	7.2
6865	Madicen, Blimbing	Medium	7.2
5086	Semarang, Demak, Godung	Bad	7.2
6866	Madioen, Tjaroeban	Bad	7.2
	Semarang, Meteseh	Bad	7.2
	Semarang	Bad	7.2
6202	Cheribon, Tjiamis	Good	7.1
5857	Pasoeroean, Kerpandjen	Good	7.1
2214	Soerabaja, Lembangboe	Bad	7.1
2358	Pekalongan, Tjomal	Bad	7.1
	Madoera, Baliga	Bad	7.1
	Semarang, Soere	Bad	7.1
2361	Pekalongan, Tjomal	Good	7.0
2360	Pekalongan, Tjomal	Good	7.0
1963	Soerabaja, Tengahan	Medium	7.0
	Batavia, Buitenzorg	Good	6.9
2359	Pekalongan, Tjomal	Good	6.9
5084	Semarang, Bonang	Bad	6.9
	Batavia, Pondoktjina	Bad	6.9
1953	Soerabaja, Madjosari	Good	6.8
	Palembang, Salatjara	Good	6.8
	Batavia, Dejpok	Bad	6.7
1573	Bantam, Sadjpera	Bad	6.7
6203	Preanger, Tjihea	Bad	6.6
	Palembang, Salatjara	Bad	3.8

relation whatsoever. Though these exceptions would seem of little importance they encouraged the author to further investigations.

After determining the titration-curves of various soils and finding their buffer-effect,[2] very distinct correlation between the buffer capacity and the productivity of the soil was found. As seen from the curves (fig. 1, 2 and 3), the soils which act as good buffers are also good soils for rice cultivation. But is the buffer curve a leading property of these soils or is it only of secondary importance?

The rice plant, like many other plants, excretes carbon dioxide, and other substances which act as amphoteric electrolytes, i.e., they can act both as bases and acids. Decaying parts of the plants act in the same way. This was demonstrated by an experiment in which 14 rice-plants about 30 cm. long were transplanted into Erlenmeyer flasks containing 75 cc. of a solution of a known hydrogen-ion concentration. The desired hydrogen-ion concentrations were obtained by adding HCl or KOH so that a series of pH values ranging from 3.0 to 9.0 results. The flasks were than placed in ordinary flowerpots covered with thick paper in which a hole was made, so that only the green parts of the plants were in the light. A corresponding series without plants served as control. At the end of one and two days, the pH volumes were determined in all the flasks. Then the solutions were renewed and the experiment carried on for one more day. The results are shown in table 2. It is obvious that the root excretions neutralized the acids or alkalies in the nutrient solution to a specific point, in this case pH 6.2.

These excretions accumulate in the layer of the soil next to the roots, and this layer then acquires special properties different from those of the surrounding soil. According to work begun by Dr. P. von der Elst on the root development of rice, several facts speak for this hypothesis. When one makes a cut through the soil and the root system of rice, there is seen around the roots a thin red layer of more highly oxidized matter than in the surrounding soil.

In soils with a low buffer content samples were taken partly from the soil surrounding the rice plant, partly from within the root system. The latter samples, when examined, had a lower pH value than the former. In one case a change took place from 7.0 to 6.7, another from 6.9 to 6.8. It therefore seems as though the plant acidifies this root layer, which results, in the case of soils with a low buffer content, in a marked change of hydrogen-ion concentration. Miyake (6) and Daikuhara (1) pointed out that the rice plant does not like even small amounts of acid, and the practical man knows that sawah rice does not thrive in humus soils, i.e., acid soils. Professor Aso of Tokio has kindly shown me a rather good rice soil (50 pikkols per bouw) which was very rich in humus. But its pH value was 7.0. According to a personal

[2] "Buffer effect" is taken to mean the soil's capacity to take up various amounts of acid or alkalies without a great change of the pH value. Some soils act as good buffers, some are bad ones.

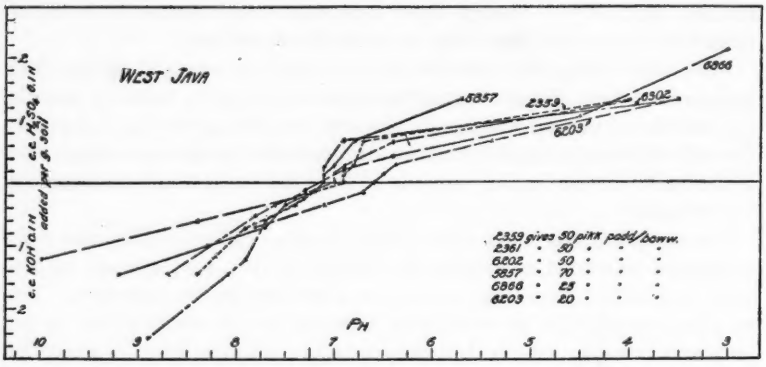

FIG. 1

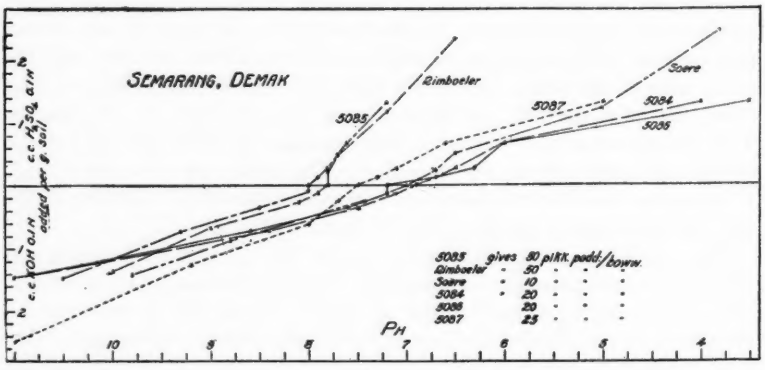

FIG. 2

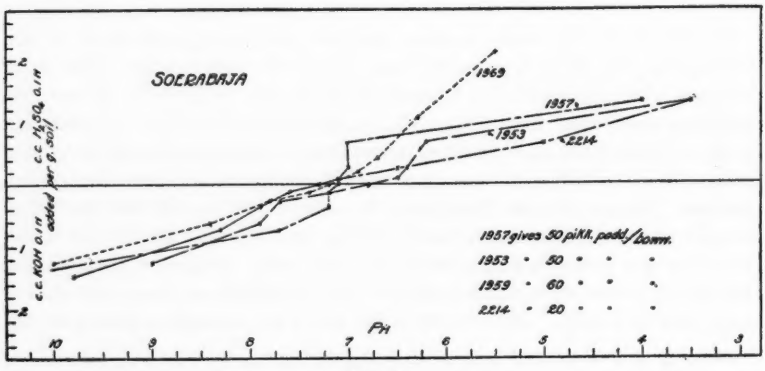

FIG. 3

CORRELATIONS BETWEEN CROP PRODUCTIVITY AND SOIL REACTION

letter from Professor Aso, green manure, and other materials rich in humus have proven very successful when applied together with lime. It seems as if the rice plant "poisons" the soil by acidifying it, but we see that this holds true only in soils which are weak as buffers or have a too low pH value. This is indeed only a secondary factor. In the acid solutions the aluminum salts are held in solution, while in neutral solutions they are precipitated. The aluminum ion has a very poisonous affect on rice (4). It is more probable that the disease is caused both by the low hydrogen-ion concentration and the aluminum. The rice plant becomes weak in such soils. and therefore falls an easy victim to different diseases.

TABLE 2

Effect of growth of rice plants in well water on its reaction

CULTURE	ORIGINAL SOLUTION			RENEWED SOLUTION
	Initial reaction	After 1 day	After 2 days	After 1 day
Control.....	3.0	3.0	3.0	3.0
Plants.....	3.0	4.5	6.2	5.0
Control.....	4.0	4.0	4.0	4.0
Plants.....	4.0	5.0	6.2	6.0
Control.....	5.0	5.0	5.0	5.0
Plants.....	5.0	6.0	6.2	6.0
Control.....	6.0	6.0	6.0	6.0
Plants.....	6.0	6.2	6.2	6.2
Control.....	7.0	6.9	6.8	7.0
Plants.....	7.0	6.1	6.1	6.0
Control.....	8.0	7.5	7.2	7.0
Plants.....	8.0	6.3	6.2	6.0
Control.....	9.0	7.8	7.5	8.0
Plants.....	9.0	6.1	6.2	6.0

What can be done to improve these bad soils? The question is hard to answer after mere laboratory experiments, but some suggestions can be made.

By adding lime, the soil can be made more resistant to acid substances for several years. The best way to apply lime is to put on small quantities at different times. In that case, the lime has a better opportunity to react with the components of the soil and will not be washed away so much by the irrigation water.

A bad soil, for instance the bad Demak, requires an application of about 2.5 tons of lime per hektare. assuming that the soil is worked to a depth of 20 cm. and that the soil, when dried at 100°C., has a volume weight of 1.5 and that it shall then have the same reaction as the good Demak.

However, in order to make the soil good for a longer period of time, a buffering substance must also be added. The best and cheapest is a good green manure crop. I am aware that much is said against humus, for, "Sawah rice can't be grown on soils rich in humus." But nearly all humus soils in Java have an acid reaction: only one out of thirty soils examined was alkaline, and this due to the fact that the place where it was found was rich in lime. It would seem therefore that it is the acid reaction which prevents rice from growing and not the fact that the soil is rich in humus. The soil which professor Aso showed me, mentioned above bears out this conclusion. By adding lime to the humus one can have a good buffer substance of the right reaction.

I take the opportunity of thanking Dr. P. von der Elst, Mr. T. T. White, and Landbouwleeraat Vink for their friendly help in taking soil samples and for their many suggestions.

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A TENTATIVE OUTLINE OF THE PLATE METHOD FOR DETERMINING THE NUMBER OF MICRO-ORGANISMS IN THE SOIL

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AND

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At the last meeting of the Society of American Bacteriologists (December, 1921), a group of soil bacteriologists came together in an informal way to discuss problems relating to the desirability of developing standard methods to be used in soil bacteriological work and to bring about a greater coöperation in the investigation of soil bacteriological problems. A committee was appointed, consisting of Dr. S. A. Waksman of the N. J. Agricultural Experiment Station, and Dr. E. B. Fred of the University of Wisconsin.

In view of the fact that the methods used in soil bacteriological work vary with almost every laboratory where that work is carried out, the committee is proposing to examine the various methods with the idea of bringing about a uniformity of the methods leading to a better understanding of the results obtained. Suggestions will be appreciated. Correspondence may be addressed to either member of the committee.

The committee is here proposing a tentative outline of the methods to be used in determining the numbers of microörganisms by the plate method. Every soil bacteriologist is urged to submit these methods to a comparative test with the methods used in his own laboratory and give the committee the benefit of his results and opinions.

I. Culture media.

1. Composition of media for determining numbers of bacteria and actinomycetes.

(a) *Sodium albuminate agar.* 1000 cc. distilled water, 1 gm. dextrose, 0.5 gm. K_2HPO_4, 0.2 gm. $MgSO_4 \cdot 7H_2O$, trace of $Fe_2(SO_4)_3$, 0.25 gm. egg-albumen dissolved in 0.1 *N* NaOH to make alkaline to phenolphthalein, 15 gm. agar.

(b) *Sodium caseinate agar.* Same as albuminate agar, except that 1 gm. of casein dissolved in 8 cc. of 0.1 *N* NaOH is used. The casein should be made according to Hammarsten or Van Slyke method. If nutrose (sodium caseinate) is used, no neutralization is necessary.

(c) *Soil extract agar and gelatin.* 1 kgm. of good fertile soil is heated with 1 liter of tap water for 30 min. in the autoclave, at 15 lbs. pressure. The turbid liquid is then poured off, a little talc is added, and the liquid filtered through a double folded filter paper, to obtain 800 cc. of filtrate. To make up 1 liter of medium, 900 cc. of distilled water, 100 cc. of this extract, 0.05 per cent K_2HPO_4, and 150 gm. of Gold Label gelatin are used. 5 gm. NaCl may be added per liter, to prevent the spread of liquefying colonies. If agar is desired, add 15 gm. instead of the gelatin.

(d) *Other media.*

2. Reaction of media—The reaction of the media should be carefully adjusted to about pH 6.8. During sterilization this medium may become more acid. After last sterilization the pH should be about 6.5.
3. Preparation and sterilization of media.
4. Media for the study of the number of fungi in the soil:

Synthetic acid medium: 1000 cc. distilled water, 10 gm. dextrose, 5 gm. peptone, 1 gm. KH_2PO_4, 0.5 gm. $MgSO_4$, 1000 cc. of distilled water, add acid to adjust pH to 4.0, 25 gm. agar.

II. Sampling of soil and preparation of dilutions:
   1. *Number of soil samples to be taken:* large composite samples of soil thoroughly mixed; at least 2 or 3 mixed samples should be used.
   2. *Number of plates to be used for each soil sample:* at least 5–10 plates for each final dilution.
   3. *Number of colonies allowable per plate (final dilution):* 50–150; the numbers obtained by the final dilutions should check within 10 per cent.

III. Incubation:
   1. *Temperature of incubation:* 28–30°C. except in the case of gelatin.
   2. *Period of incubation:* at least 8 to 10 days for bacteria; 14 days for actinomycetes; 2 to 3 days for fungi (special medium). Incubate plates under bell jar or tin cans to prevent evaporation.

IV. Counting of plates

V. Mathematical interpretation of results

VI. Maximum error to be allowed for an accurate count

VII. Types of colonies on the plate: Molds, Actinomycetes, Yeasts, Chromogenic forms, *Mycoides*, *Fluorescent*, *B. subtilis*, *B. vulgatus*, *B. coli*, etc.

MOVEMENT OF LEGUME BACTERIA IN SOIL[1]

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A study of the amount and rate of movement of legume bacteria in soil is of practical importance from the standpoint of inoculation. It would at first seem probable that the successful inoculation of most of the legume plants throughout a field would mean the subsequent inoculation of the remaining plants in the field. If this were true, a very light inoculation of a field or of a lot of seed would be almost as effectual as a heavier inoculation. In practice this has been found untrue, for in field tests where inoculated and uninoculated plants are grown in adjacent rows, the uninoculated plants rarely show nodules. Then, after the nodules have broken up and decayed, there is the question of how much and how fast the legume bacteria will move through the soil.

That the soil is an effective filter for removing organisms has long been established; and the fact is made use of in water purification. This filtering action is particularly noticeable where the soil about a legume is inoculated at the surface with a suspension of the proper legume bacteria. In this case only the upper part of the root system produces nodules. This was observed as early as 1892 by Nobbe, et al. (1) and has been often observed by the writers. These investigators also inoculated sand at a depth of 8 inches and found that only the deeper roots were inoculated. Their conclusion was that legume organisms do not diffuse readily through the soil. Hunter (2) showed that this filtering effect varied with the kind of soil and the depth of the soil.

Kellerman and Fawcett (3) worked with *B. radicicola*, *B. ochroceus* and a *paracolon* organism in sterilized favorable soils saturated with water. They observed that the organisms progressed horizontally at the rate of about 1 inch in 48 hours. In barely moist soils the rate of movement of *B. radicicola* was reduced to about 1 inch in 72 hours. These exepriments were conducted at a temperature of 25°C. When the temperature was 10°C. the rate of progress of *B. radicicola* was only about 1 inch in 3 days in saturated soils. The progress was assumed to be due to growth of the organisms.

Ball (4) planted bacteria-free alfalfa and bur clover seeds in boxes of sterilized soil. The soil was watered by a tube that ran full length along the bottom of the box to avoid water currents as much as possible. The soils were inoculated at one end of the boxes with a suspension of legume bacteria, made by crushing nodules of alfalfa or bur clover in water. He concludes that *B. radicicola* diffuses through the soil, if the soil has the proper moisture content. He suggests that the movement is due to currents in the soil water, aided by the motility of the organism and its rapid proliferation. He agrees with Kellerman that the rate of movement is about 1 inch in 48 hours. From a group of similar experiments Hunter (2) drew practically the same conclusions: that the movement of legume bacteria in soil is chiefly a result of water currents. He agreed with Kellerman and Fawcett that a high moisture content in the soil was necessary for an appreciable movement of the legume bacteria.

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[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

In the experiment outlined below the apparatus was modified so as to cut down to a still greater extent the movement of soil water.

Two galvanized iron boxes 24 x 7 x 7 inches were filled with 5 inches of limed yellow sand. Each box was divided longitudinally by a metal wall, the lower part of which was perforated. This divided the box into a main compartment in which the plants were grown, and a small side compartment, used to receive the water or nutrient solution for the soil. This side compartment was also filled with fine sand. Hence all the water before it reached the plants passed through 6 inches of sand and the perforated division wall. Boxes and sand were heated in the autoclave for 2 hours at 15 pounds, pressure. Soybean seeds were treated with mercuric chloride. In box 1 eight mammoth yellow soybean seeds were planted at 6- or 7-inch intervals; and in box 2 nine soybean seeds were planted at similar intervals. Six days later, when the seedlings had just begun to leaf, an area which extended from the end of the box to the stem of the first soybean plant, within the partition, was inoculated with a heavy suspension of soybean bacteria, a mixture of four tested strains. Thus in box 1 the area wet by the inoculating fluid extended 5 inches from the end, in box 2, 6.5 inches from the end.

Thereafter the sand was kept at approximately three-fourths saturation by watering into the smaller side compartment. Thus, the water entered the larger compartment from the side and below, reducing the possibility of the legume bacteria being transported down the length of the box by water currents. Hopkins and Pettit nutrient solution was also added in this way.

Thirty-five days after inoculation the sand was carefully washed from the roots of the soybean plants, with special precautions not to change the arrangement of the roots. As each layer of sand was washed off, the newly uncovered roots were examined for the presence of nodules. In this way the exact position of all the nodules was ascertained; and in particular the position of the nodules farthest away from the place of inoculation was noted. Similarly, after 54 days the sand was washed from the roots of the plants in box 2.

In both cases the roots of the first four plants had nodules, while the roots of the remaining plants had none. The striking thing, however, was that in both boxes all nodule formation had taken place within a sharply defined zone which included the soil within 6.7 inches from the inoculated end of the boxes. Within this 6.7 inches all the roots were well inoculated; beyond the 6.7 inche mark there were no nodules even on roots of the same plant. This is graphically shown in the accompanying photographs of the second, third, fourth and fifth plants in a box. The roots are arranged just as they were in the soil. The roots of the second plant were entirely within this inoculated area and showed an even distribution of nodules. The roots of the third plant were only half within this area and have nodules accordingly. Two roots from the fourth plant passed the top root of the third plant and reached into the inoculated area and had nodules where they entered that area. The roots of the fifth plant were all outside of the area and hence had no nodules.

This would mean then, that the legume organisms had travelled or been carried at the rate of about 0.1 or 0.2 inch per day in this experiment.

This work, then, substantiates that of the investigators cited above, and shows that where the movement of soil water is minimized the rate of movement of the legume bacteria as found is still less than that previously determined.

A field test at this station has demonstrated on a large scale the slow rate of movement of legume bacteria. A small plot of virgin soil on the university farm was planted to Ito San soybeans. The surface of the plot was far from level, with a decided slope towards one end. The soybeans were planted in hills, 3 feet apart in each direction. It was arranged so that inoculated and uninoculated beans were sown in alternate hills. In one row the inoculted seed were planted in the hills of even numbers, while in the adjoining row the odd numbers were planted with the inoculated seed. The diagram shows the scheme followed in planting.

O X O X O X

X O X O X O

O X O X O X

X O X O X O

O X O X O X

X O X O X O

O = Uninoculated
X = Inoculated

Inoculation was accomplished by applying directly to the seed a pure culture of bacteria. The treated seed was shown as soon as possible after inoculation.

About three months after seeding, just as the pods were formed, several plants from various parts of the plot were dug up carefully and examined for nodules. In every case, roots from the inoculated hills were thickly studded with nodules, while those from the untreated hills were free of nodules. Only one exception was found; here the uninoculated roots showed one or two nodules. When examined again two weeks later the effect of inoculation on nodule production was even more pronounced. The untreated plants were without nodules. Although but one experiment has been made, it seems safe to conclude that in soil of this Miami silt loam type, soybean bacteria spread slowly if at all through soil, unless carried by the host plant or by wind, rain, etc.

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PLATE 1

FIG. 1. SECOND PLANT FROM INOCULATED END OF BOX
FIG. 2. THIRD PLANT FROM INOCULATED END OF BOX

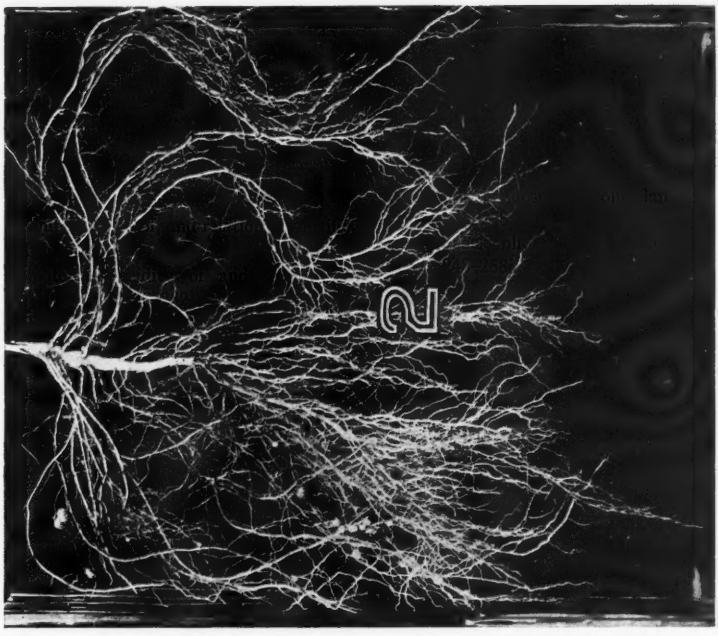

FIG. 2

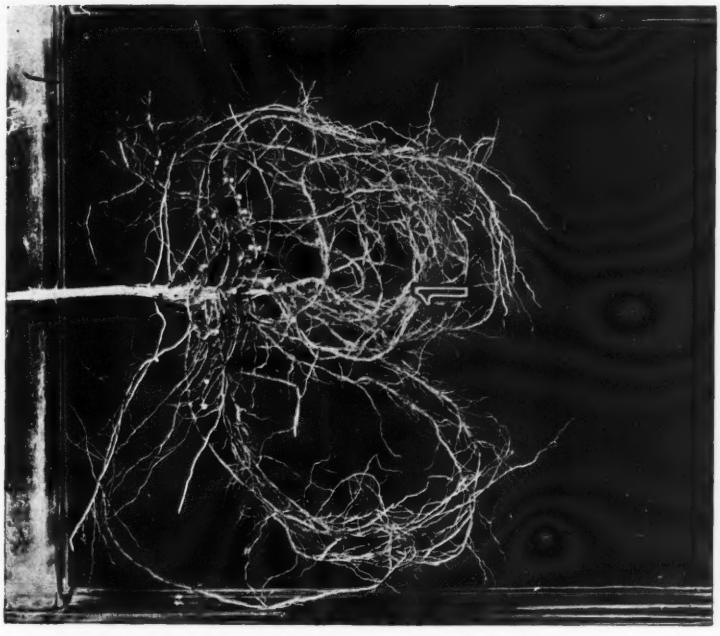

FIG. 1

PLATE 2

FIG. 3. FOURTH PLANT FROM INOCULATED END OF BOX
FIG. 4. FIFTH PLANT FROM INOCULATED END OF BOX

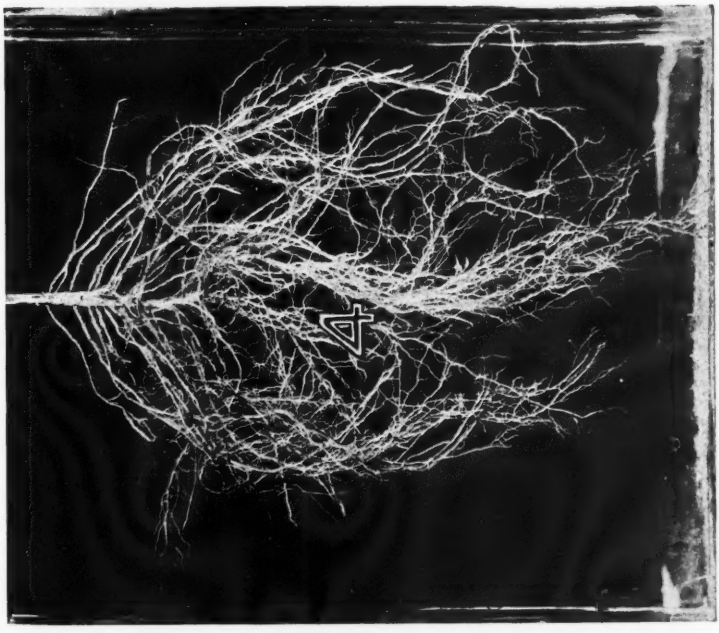

FIG. 4

FIG. 3

COMPOSTING ROCK PHOSPHATE WITH SULFUR IN SLIGHTLY ALKALINE CALCAREOUS SOILS[1]

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Received for publication October 18, 1921

INTRODUCTION

The studies made by Lipman, McLean and Lint (10, 11) on the effect of oxidation of sulfur in soils upon the solubility of rock phosphate led to the positive conclusion that the method of composting rock phosphate with sulfur could be made practical and moreover would have an advantage over the old methods of making phosphoric acid rapidly available for plant food.

In continuing their experiments they concluded that mixtures of soil, rock phosphate and sulfur for plants grown in pot cultures compare favorably in value with commercial products. Since then several investigators have conducted experiments in the same field.

The purpose of the work of Lipman and his associates at the start was to produce a compost mixture which could easily be made by farmers. As soon as laboratory experiments are brought to the farm a number of difficulties arise, such as methods of mixing, the optimum water-content of the composts during incubation, proportion of soil, temperature, and the length of time required before the mixture is ready for use. When such general laboratory experiments are being duplicated in the factory still more problems have to be solved, which do not concern the workers in the Agricultural Experiment Stations.

One of the main problems is the time required before the cultures begin to make the tri-calcium-phosphate available for plant food. McLean found in his best cultures that after 15 weeks small amounts of phosphorus became available. The recently reported experiments by Lipman and Joffe (9) show, however, that under favorable conditions, as an abundance of soil, high initial acidity of the soil used, excess of sulfur, and rather high temperature this time may be considerably reduced. To duplicate these favorable conditions on a large scale a number of difficulties have to be overcome.

Lindet and Bruno (8) doubted that sulfur oxidizing organisms in compost mixtures of rock phosphate, sulfur and a *calcareous* soil could produce sufficient acid to dissolve the phosphates, for "the acid formed would by preference attack the calcium carbonate and not

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[2] Part of a thesis submitted to the faculty of Rutgers College and the State University of New Jersey in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express here his thanks to Dr. J. G. Lipman for the opportunity to carry on these studies in France, to Dr. André Helbronner, formerly chef de laboratoire in the Sorbonne University at Paris for his cordial encouragements throughout the period of investigations, and to Monsieur J. Gavelle, Directeur de la laboratoire de récherches des Établissements Kuhlmann à Paris for the help rendered in analyzing the numerous samples.

the tri-calcium phosphate." The opportunity has been given to the writer to make a study of these and other problems in France where the greatest part of the soils are calcareous. Later (13) it was found that sulfur organisms would oxidize sulfur in the presence of "alkali" salts.

PURPOSE OF THE WORK

The work undertaken dealt mainly with the problem of how to reduce the first part of the incubation period in mixtures of soil rock phosphate and sulfur mixtures before the phosphorus becomes available when a *calcareous, slightly alkaline soil* is used, and of reducing the amount of such a soil in the mixture to a point which would make the process practical in the factory.

A study was made of the following factors:

1. Influence of temperature on the activities of the sulfur-oxidizing organisms.
2. Influence of light.
3. Partial sterilization of the mixtures.
4. Effect of stimulants.
5. Initial reaction.
6. Aeration.
7. Replacement of soil by other substances as a source of nitrogen.
8. Reduction of the proportion of sulfur in the mixtures.

METHODS

The composts were made by thoroughly mixing air-dry portions of soil, rock phosphate, sulfur, and other materials used, if not otherwise stated, and were then placed in tumblers covered with glass plates. Each compost was weighed and the water-holding capacity determined on portions of it according to the Hilgard (6) method. Samples were weighed out and the relative acidity, hydrogen-ion concentration and citrate-soluble phosphoric acid determined. The mixtures were then inoculated with soil extract known to contain the sulfur-oxidizing organisms. The compost mixtures were incubated at 30°C. and at laboratory temperature. Daily records taken of the room temperature showed a fluctuation of the temperature between 10°C. and 21°C., the average temperature being 17°C. The composts were kept at 60 per cent of the water-holding capacity throughout the period of incubation. The amount of water lost by evaporation, determined by placing the tumblers on the scale pan, was added once each week to the composts kept at room-temperature and twice each week to the composts incubated at 30°C. Each time, after the water was added, the mixtures were removed from the tumblers and thoroughly mixed to provide for sufficient aeration, and to break up the small aggregates formed.

Citrate-soluble phosphoric acid was determined by sifting the air-dry sample through a 50-mm. sieve. Two grams of this material was finally ground in a cobalt mortar with a total amount of 80 cc. ammonium citrate (400 gm. per liter), decanted into a 200-cc. flask under repeated grinding. The remainder of the material in the mortar was washed into the flasks with distilled water. The stoppered flasks were shaken and left standing for 15 hours at room temperature. They were then kept for 1 hour on a water bath at 40°C., cooled, made to volume, shaken and filtered. An aliquot portion was precipitated with $MgNH_4Cl$, and washed five times with ammonia water (4 per cent). The filter with contents was left standing for a few hours to dry, transferred to a platinum crucible, ignited (placed at a blow flame till weight was constant), cooled and weighed as $Mg_2P_2O_7$.

The relative acidity was determined by transferring an air-dry weighed sample to 200-cc. flasks with 160 cc. of boiling water, thoroughly shaking every 5 minutes throughout a period

of ½ hour, cooling flasks and contents, restoring volume and shaking again. After settling, an aliquot was drawn off, boiled to expel carbon dioxide, cooled and titrated with 50 *N* sodium hydroxide. Phenolphthalein was used as indicator and the liquid titrated until a faint pink color remained. The results in the tables, however, are given as 0.5 normal sodium hydroxide.

The water extracts for the determination of the hydrogen-ion concentration were prepared according to the method of Gillespie (5). The hydrogen-ion concentrations as expressed in pH values of the extract were determined by the colorimetric method as recommended by Clark and Lubs (3). Duplicate determinations of the hydrogen-ion concentrations, but not of relative acidity nor of the phosphoric acid were made, except when the results did not agree with the constructed curves for the relation between acidity and available phosphoric acid.

From time to time sulfate determinations were made to check up with the relative acidity.

PLAN OF EXPERIMENT

Several sets of experiments were arranged in a similar way and according to the following plan:

The proportions of the mixtures were:

100 gm. soil
120 gm. sulfur
400 gm. rock phosphate

Soils used were slightly alkaline, calcareous clayey silt loams; the water extract requiring of 2–4 cc. 50 *N* hydrochloric acid per 100 gm. for neutralization and having pH values of 7.1 –7.6. These soils were poor in organic matter; and their water-holding capacity was but 24 and 26 per cent.

The flowers of sulfur were from the Italian mines.

The rock phosphate used was Tunisian rock containing 25.99 per cent of total phosphoric acid and 56.79 per cent of total tri-calcium phosphate.

The water-holding capacity of the soil-sulfur-rock phosphate mixtures was 20 and 22 per cent.

EXPERIMENTAL RESULTS

Series I. Influence of light, temperature and peptone

Until the experiments reported below were begun nothing was known about the influence of temperature on the production of available phosphoric acid.

Since then Shedd (16) has reported greenhouse experiments on the availability of phosphoric acid in compost mixtures and he concludes from his study that high temperature exerts a decided influence on the sulfate production and the available phosphorus.

McLean (12) states that when peptone was added to the mixtures "sulfur was largely transformed into sulfites." He laid emphasis on the fact that peat, manure and peptone should not be added to the mixtures for "a compost is more efficient in the absence of large amounts of organic materials." Brown and Gwinn (1, p. 369–389) showed in their experiments that more available phosphoric acid is produced where manure is included in the compost. Ellett and Harris (4) in their recently published work came to the same conclusion. It should be mentioned here that McLean used soils with sufficient organic matter, especially where he used rich greenhouse soils.

In earlier work it was demonstrated by several investigators that various soil types are not alike in their ability to render rock phosphate soluble. At the suggestion of Dr. Lipman compost experiments were carried on in different localities with different soil types. Aside from the "power" of soils to produce sulfates, which according to the results obtained by

Shedd (15), Ellett and Harris (4), and Brown and Kellogg (2, p. 49–111) varies with the soil, the addition of peptone to a soil poor in nitrogenous material could therefore throw light upon the problem of whether or not nitrogen in sufficient quantities is necessary for the activities of the sulfur organisms.

The experimental results of this series consist of the citrate-soluble phosphoric acid made available during an incubation period of 22 weeks, the relative acidity produced, and of the hydrogen-ion concentrations of these mixtures expressed in pH values at definite intervals during this period.

In order to determine what the effect of light on the activities of the sulfur organisms might be, two sets of quadruplicate tumblers were placed on a laboratory table and two similar sets were kept in a dark closet underneath the table. To duplicates of these sets was added peptone equivalent to 3

TABLE 1

Influence of temperature, light and organic material on the production of acidity and available phosphoric acid in compost mixtures

NUMBER	TREATMENT	INITIAL DETERMINATIONS			AFTER 22 WEEKS		
		Reaction		Citrate-soluble P_2O_5	Reaction		Citrate-soluble P_2O_5
		*alk., cc.**	*pH*	*per cent†*	*cc.°*	*pH*	*per cent†*
1	In darkness, laboratory temperature.	0.04	7.2	0.0	73.3	4.8	0.40
2	In darkness, laboratory temperature, 3 per cent peptone.	0.04	7.2	0.0	220.7	3.0	9.31
3	In light, laboratory temperature.	0.04	7.2	0.0	45.8	4.9	0.31
4	In light, laboratory temperature, 3 per cent peptone.	0.04	7.2	0.0	193.2	3.1	8.72
5	In darkness, 30°C.	0.04	7.2	0.0	132.1	3.2	5.24
6	In darkness, 30°C., 3 per cent peptone.	0.04	7.2	0.0	278.0	3.0	18.92

* Alkalinity expressed in cc. of 0.5*N* H_2SO_4

† Total P_2O_5 content in mixture was taken as 100 per cent.

° Acidity expressed in cc. 0.5 *N* NaOH required to neutralize 100 gm. air-dry compost.

per cent of the soil mixtures for the purpose of studying the influence of nitrogenous matter. Two other duplicate sets were placed in an incubator at 30°C. of which two mixtures received in addition a similar proportion of peptone. The results obtained at the end of 22 weeks are given in table 1.

The relative acidity, hydrogen-ion concentration and available phosphoric acid increased in all cases regularly, which when plotted formed similar curves. The quantities of acids or acid salts increased continuously, but the intensity of the acid as indicated by the pH values remained the same after a certain point was reached, the acid being neutralized by the tri-calcium phosphate. If more sulfur had been applied the strength of the acid produced would presumably have been greater, but since only a small theoretical excess of sulfur was given and the cultures were not carried on till greater amounts of tri-calcium phosphate were made available, the pH values remained at from 2.9 to

3.0. Determinations of hydrogen-ion concentrations made at the end of every week showed slight differences from week to week in pH values, namely, 2.9 to 3.2.

The influence of temperature was very marked throughout the time of experimentation, the higher temperature causing a much higher relative acidity and consequently producing more available phosphoric acid. Even in the mixtures to which peptone was added the accumulation of soluble P_2O_5 was twice as great when the compost was incubated at 30°C. (No. 6) as when they were incubated at room temperature (No. 2).

The nitrogenous material introduced in the form of peptone in this calcareous soil which was poor in nitrogen, had a still greater influence. Culture 5 kept at 30°C. had, after 22 weeks, but 5.2 per cent of the total P_2O_5 made citrate-soluble and had accumulated an acidity equivalent to 132.1 cc. 0.5 *N*

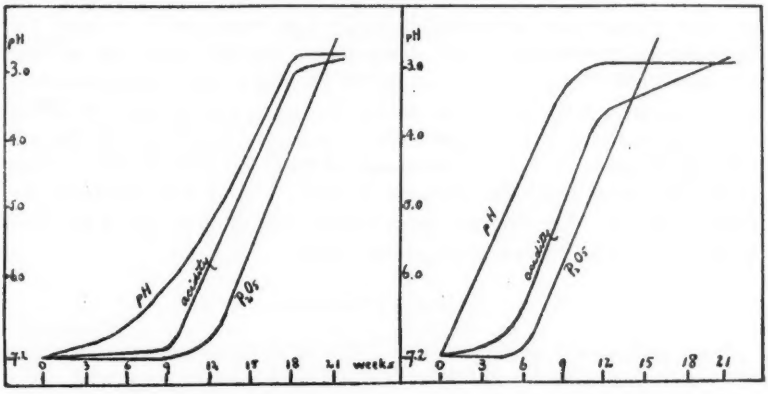

FIG. 1. ROOM TEMPERATURE FIG. 2. 30° CENTIGRADE

Influence of temperature upon the change of hydrogen-ion concentration and accumulation of acidity and available phosphoric acid.

NaOH per 100 gm. mixture, while culture 2 kept at room temperature and to which peptone was added showed 9.3 per cent of the total P_2O_5 made available, and an acidity equivalent to 220.7 cc. 0.5 *N* NaOH. This explains at the same time why additions of manure in certain cases, as found by other investigators, render more phosphoric acid available. The sulfur-oxidizing organisms need a certain quantity of nitrogen to carry on their activities. The source of the nitrogen does not necessarily have to be of an organic origin as is shown in another part of this study. When the amounts of nitrogen in the soil are sufficient, additions of nitrogenous materials become detrimental as is pointed out by McLean.

From this series it is evident that calcareous slightly alkaline soil does not inhibit the activities of the sulfur-oxidizing organisms either from attacking the tri-calcium phosphate or transforming it into a soluble form as had been

supposed by Lindet and Bruno. In all cultures there was an accumulation of sulfates as expressed in terms of acidity, even in the cultures kept in the light at laboratory temperature and without additions of peptone.

The influence of light was noticeable although not very pronounced. Diffused light in factories might be somewhat detrimental, but presumably very slightly if conditions were more favorable than those prevailing in this experiment.

From a number of cultures, curves were constructed to bring out the relation between the accumulation of acidity, pH values, and quantities of soluble phosphoric acid produced. These relations are shown graphically in figures 1 and 2. The curves in figure 1 are constructed from mixtures incubated at toom temperature, and the curves of figure 2 for the cultures incubated at 30°C. These two sets of curves show clearly the influence of temperature upon the activities of the sulfur-oxidizing organisms. Cultures incubated at room temperature accumulated acidity and consequently changed the hydrogen-ion concentration very slowly during the first weeks, but cultures incubated at 30°C. commenced to change the reaction of the mixtures instantly at the beginning of the incubation period, the rate of accumulation of acidity decreasing in the same way when a certain point is reached both for the cultures incubated at room temperature and for those incubated at 30°C. That part of the curve represents the point at which most of the phosphorus becomes available. The curves of figure 2 show that the amounts of free acid increased with the increase of the temperature.

Series 2. Stimulation

In the work reported by McLean (12) a number of salts and organic substances were added to the compost mixtures in order to determine which salt or substance would exert a stimulating action on the sulfur oxidation processes. He found that all salts used, except, under certain conditions, ferrous sulfate and aluminum sulfate and a combination of the two salts, had no influence on the production of available phosphoric acid. Since sulfuric acid and acid sulfates are produced by the organisms it was thought that small amounts of H_2SO_4 added at the beginning would possibly stimulate their activities.

The soil used in this series consisted of an equal mixture of two different fine calcareous silt loams, which were slightly alkaline, their water extracts having pH values of 7.4 and 7.5.

In one series the sulfuric acid was added before the mixtures were inoculated with the soil infusion and in another series immediately afterwards. The two series checked very closely and the results obtained from the first are given in table 2. At the end of the first week peptone equivalent to 3 per cent of the soil used was added to all series. All mixtures were incubated at room temperature. Determinations of acidity, hydrogen-ion concentrations and citrate-soluble P_2O_5 were made at the end of every three weeks, but only the data obtained at the beginning and at the end of 18 weeks are included as representative figures of this series.

During the first eleven weeks cultures 1 to 7 particularly seemed more or less stimulated by the treatment as compared with the check cultures, but they were gradually overtaken by the checks in acidity accumulation, till after 18 weeks several of the cultures were behind the checks. Cultures 2, 3 and 4, however, had at that time a still higher degree of acidity and also more available phosphoric acid than had the check cultures. From these and other experiments, the conclusion can be drawn that when a fine calcareous silt loam is used, H_2SO_4 exerts a stimulating influence during the first part of the incubation period if quantities of approximately 7 to 10 liters (52° Bé) per ton are used, but depresses the activities of the sulfur-oxidizing organisms when larger amounts are added.

Several salts were tried out in the earlier work by McLean as possible catalytic agents. Some organic acids and two different acid salts which

TABLE 2

Influence of sulfuric acid in compost mixtures when a fine calcareous alkaline soil is used

NUMBER	1.0 *N* H_2SO_4 APPLIED PER 100 GM. OF MIXTURE	INITIAL DETERMINATIONS			AFTER 18 WEEKS		
		Reaction		Citrate-soluble P_2O_5	Reaction		Soluble P_2O_5
	cc.	*alk., cc.**	*pH*	*per cent†*	*cc.°*	*pH*	*per cent†*
1	1.8	0.05	7.1	0.00	140.4	3.6	9.65
2	3.8	0.04	7.1	0.00	165.5	3.5	10.40
3	5.8	0.06	7.1	0.00	197.3	3.4	12.10
4	6.8	0.01	7.0	0.00	160.2	3.2	11.41
5	7.8	0.00	6.9	0.10	126.1	3.5	9.15
6	9.8	0.01	7.0	0.08	90.6	3.6	8.80
7	11.8	0.00	6.8	0.93	87.4	3.6	7.17
8	13.8	0.02	7.0	1.13	78.4	3.6	4.65
9	None	0.04	7.2	0.00	147.8	3.3	9.98

* † ° Same as table 1.

suggested themselves for several reasons had not been employed. As organic acid for our experiment acetic acid, which could possibly lower the alkalinity of the mixtures, was chosen and as acid salts, sodium bi-carbonate as a possible source of carbon di-oxide and sodium bi-sulfite.

These constituents were added in small quantities to mixtures as previously described, but no additions of sulfuric acid were made. Treatment of the mixtures and the results obtained after 12 weeks are reported in table 3.

Acidity and hydrogen-ion concentrations were determined at the end of every two weeks, but are not reported here since these data were merely used in checking up the procedure of the process of sulfate production.

The cultures to which acetic acid was added ran in duplicate very inconsistently, but no stimulation could be noticed.

There was a gradual increas of acidity in all mixtures, but although a high relative acidity was reached in cultures 5, 6, and 7, the intensity of the acid

produced as indicated by the low hydrogen-ion concentration seemed less. Nevertheless, a marked stimulating effect was to be noticed throughout the entire period of the experiment in the production of acidity and citrate-soluble phosphoric acid. This might have been due to a production of CO_2 (probably needed by the sulfur organisms for greater acitivity) when other bacteria were depressed to a high degree in these acid media.

The cultures to which sodium bisulfite in solution was added as well as the cultures with acetic acid were behind the checks. The influence of $NaHSO_3$ was depressing rather than stimulating for these soil types.

Two series of cultures were made up of the same constituents as described under series 1, and kept at toom temperature, one in light and the other in

TABLE 3

Influence of acetic acid and sodium bicarbonate and sodium bisulfite in composts kept at room temperature

NUMBER	TREATMENT	INITIAL REACTION		AFTER 12 WEEKS		
				REACTION		Citrate-soluble P_2O_5
		*alk., cc.**	*pH*	*cc.°*	*pH*	*per cent†*
1	None	0.04	7.2	103.6	3.3	6.69
2	2 cc. 1.0 *N* CH_3COOH	0.02	7.1	71.4	4.8	0.83
3	6 cc. 1.0 *N* CH_3COOH	0.02	7.0	75.8	4.1	6.34
4	10 cc. 1.0 *N* CH_3COOH	0.01	7.0	73.0	4.1	4.32
5	0.01% $NaHCO_3$	0.06	7.3	187.9	4.8	13.94
6	0.02% $NaHCO_3$	0.05	7.2	186.9	4.7	11.43
7	0.03% $NaHCO_3$	0.05	7.2	183.4	4.7	12.05
8	0.10% $NaHSO_3$ (40-% sol.)	0.04	7.3	81.1	5.0	5.10
9	0.20% $NaHSO_3$ (40-% sol.)	0.04	7.3	82.4	5.0	6.01
10	0.30% $NaHSO_3$ (40-% sol.)	0.04	7.3	84.1	5.2	4.70
11	0.50% $NaHSO_3$ (40-% sol.)	0.04	7.3	70.3	5.3	4.52
12	None	0.04	7.1	104.4	3.4	7.01

* † ° Same as table 1.

darkness. Two other series at 30°C. were similarly treated. One tumbler in each series was treated with varying amounts of sulfuric acid (see table 2), another with sulfuric acid and a mixture of .02 per cent $FeSO_4$ and .02 per cent $Al_2(SO_4)_3$, and still another with the iron salts but no sulfuric acid. From the data obtained it was evident that if stimulation was caused by the iron salts it was not great enough to be out of the range of the experimental error.

Series 3. Partial sterilization.

It had been shown in the experiments of Lipman, McLean and Lint (11) that sterilization of the mixtures was detrimental to the production of available phosphoric acid. They therefore drew the conclusion that sulfur oxidation was a biological process. No attempt was made to study the influence of partial sterilization by means of salts or acids. The investiga-

tions of Russell (14) with soils treated with antiseptics showed an increase in bacterial numbers as the result of improvement of the soil medium. This worker used carbon disulfide and came to the conclsion that although the bacterial numbers were increased, partial sterilization did not improve the bacterial flora. This was shown by the fact that the old flora, when reintroduced into partial sterilized soil, attained higher numbers and effected more decomposition than the new flora. Partially sterilized soil plus 0.5 per cent of untreated soil, or an unfiltered aqueous extract of untreated soil, soon contained higher bacterial numbers per gram and accumulated ammonia at a faster rate than partially sterilized soil alone. Truffaut (17) concluded after repeated trials that calcium sulfide was an exceptionally good means of partial sterilization. Hutchinson (7) studied the effect of caustic lime for partial sterilization as practical means and obtained good results. Many other investigators worked with different kinds of antiseptics like phenol, naphthaline, toluene, etc., which seemed less suitable for the purpose of this study. The purpose of partial sterilization in the case of the studies at hand would be to suppress undesirable bacterial growth in the cultures and thereby favoring the development and perhaps multiplication of the sulfur-oxidizing organisms.

On account of the relatively high calcium content of the soils used and the abundance of calcium in the rock phosphate used, and in view of the necessary production of acidity, caustic lime was not used. The excellent results obtained by Truffaut in the treatment of soil with calcium sulfide as a means of partial sterilization led the writer to conduct a series of experiments in which the cultures received different amounts of calcium sulfide. In these preliminary studies it was brought out that pure calcium sulfide and the technical product in H_2SO_4 did not produce satisfactory results. The acidity obtained in compost mixtures after 6 weeks of incubation with a neutral garden soil (incubated at 28°C.) was:

Nothing added, 98.4 cc. 0.5 *N* NaOH per 100 gm. mixture.
CaS added, alkaline.
CaS+H_2SO_4 added, 12.1 cc. 0.5 *N* NaOH per 100 gm. mixture.

For the partial sterilization studies, the same soil mixtures and the same proportions of sulfur and rock phosphate were used as in series 2. The compost mixtures were kept at room temperature throughout the incubation period. The results obtained with different amounts of NaCl per 100 gm. of mixture are recorded in table 4.

From these data it is readily seen that NaCl added in small quantities depressed the growth and activities of the sulfur oxidizing organisms considerably. Even where but .01 per cent NaCl was added the production of relative acidity after 13 weeks was less than in the check cultures and the available phosphoric acid was less than half the amount produced if compared with the cultures which received no additions of NaCl. Incidentally it was shown that soils secured near the sea coast and containing small amounts of chlorides were not suitable for rapid production of acidity and available P_2O_5. Bacterial counts showed that although fewer species of soil bacteria were present and smaller numbers existed, the sulfur-oxidizing organisms were not favored by their absence. Influence of chlorides on sulfur oxidation by microörganisms was also noted in studies with black alkali soils from California (13).

The same soil-sulfur-rock phosphate mixtures were used for cultures to which 0.1 *N* HCl was added. Additions varied from 4.5 cc. to 88 cc. per 100 gm. mixture. The compost mixtures were incubated for the first 6 weeks at 30°C. and then placed in a dark cupboard at room temperature. Water was added as usual and the mixtures stirred at the end of each week. Relative

TABLE 4

Influence of partial sterilization on the production of acidity and available P_2O_5 in compost mixtures incubated at room temperature

NUMBER	TREATMENT WITH NaCl	INITIAL REACTION		AFTER 13 WEEKS		
				REACTION		Citrate-soluble P_2O_5
	per cent	*alk., cc.**	*pH*	*cc.°*	*pH*	*per cent†*
1	0.01	0.04	7.2	65.6	3.9	1.82
2	0.02	0.04	7.2	57.2	4.9	trace
3	0.03	0.04	7.3	55.7	4.9	0.83
4	0.01	0.08	7.3	55.4	4.9	trace
	9 cc. 0.1 *N* HCl added					
5	Check	0.04	7.2	79.0	3.4	4.15

* † ° Same as table 1.

TABLE 5

Compost mixtures with additions of varying amounts of HCl for partial sterilization

NUMBER	0.1 *N* HCl ADDED PER 100 GM. MIXTURE	INITIAL DETERMINATIONS			AFTER 14 WEEKS		
		REACTION		Citrate-soluble P_2O_5	REACTION		Citrate-soluble P_2O_5
	cc.	*cc.°*	*pH*	*per cent†*	*cc.°*	*pH*	*per cent†*
1	4.5	0.04	6.8	0.00	66.9	4.9	2.36
2	9	0.10	6.6	0.00	68.4	4.8	2.13
3	14	0.10	6.6	0.00	68.0	4.8	0.77
4	18	0.16	6.7	0.00	64.4	4.8	0.76
5	28	0.12	6.7	0.00	*lost*	...	...
6	38	0.15	6.7	0.00	27.2	5.6	0.61
7	48	0.16	6.7	0.00	20.9	5.8	0.68
8	58	0.50	6.2	0.00	10.1	5.8	0.46
9	68	6.31	5.3	0.68	5.3	6.4	0.19
10	88	7.70	5.2	0.40	5.5	6.4	0.00
11	check	alk.* 0.04	7.3	0.00	104.2	3.2	5.22

* † ° Same as table 1.

acidity and pH values were determined every time when stirred. Most of the free acid had been neutralized by the tri-calcium phosphate, but according to the low pH values considerable free acid was still present at the start of the experiment, especially cultures 9 and 10, as recorded in table 5.

After one week of incubation the pH values had gone up and approached the neutral point. There was but slight change at the end of the third week

and culture 8 was still slightly alkaline. At the end of 14 weeks all cultures to which HCl was added were far behind the check cultures, in relative acidity as well as in available P_2O_5. Bacterial activities had been very slight in cultures 9 and 10, and the small amounts of P_2O_5 available at the beginning of the experiment were transformed into insoluble phosphates. Even where but 4.5 cc. 0.1 *N* HCl per 100 gm. mixture was added it proved strongly detrimental. The results obtained confirmed the conclusions drawn from the previous experiment that chlorides are detrimental to the activities of the sulfur-oxidizing organisms.

TABLE 6

Influence of varying amounts of soil on the production of acidity and soluble P_2O_5 in composts containing 120 gm. sulfur and 400 gm. rock phosphate, incubated at 30°C.

NUMBER	QUANTITY OF SOIL USED	INITIAL REACTION		AFTER 15 WEEKS		
				REACTION		Citrate-soluble P_2O_5
	gm.	*alk., cc.**	*pH*	*cc.°*	*pH*	*per cent†*
1	25	0.08	7.2	166.6	2.9	9.12
2	50	0.08	7.2	193.6	2.9	12.90
3	75	0.08	7.3	199.3	2.9	13.82
				After 11 weeks		
4	80	0.08	7.3	227.7	2.9	14.10
5	90	0.07	7.3	232.7	2.9	14.00
6	100	0.08	7.3	216.6	2.9	13.92

* † ° Same as table 1.

Series 4. Replacement of soil by other substances as a source of nitrogen

In his studies McLean (12) came to the conclusion that 100 parts of soil, 120 parts of sulfur and 400 parts of rock phosphate would be the most economical combination for the production of available phosphoric acid. All investigators used great quantities of soil, based on the work of McLean. Some of them increased the amounts of soil or added manure. No attempt had been made to reduce the quantities of soil and replacing it by other material as sources of nitrogen except in the studies of pure cultures which were made in the laboratory of the New Jersey Agricultural Experiment Stations, which studies were made mainly during the absence of the writer.

For practical purposes a great bulk of soil in the mixtures is undesirable. The high cost of handling and transportation are regarded as considerable items, aside from the problems of mixing and storing.

A series of experiments was conducted with a rather rich, slightly alkaline, calcareous garden soil. All mixtures were placed in an incubator at 30°C.

Table 6 includes a part of the data secured.

Determinations of citrate soluble P_2O_5 made after 7 weeks showed that cultures 4 and 5 had made more phosphoric acid available than the cultures with 25 parts of soil after a period of 15 weeks. Cultures with 80 or more

parts of soil had as much or more available P_2O_5 in 11 weeks as cultures with 75 or less parts of soil in 15 weeks. The difference in relative acidity between cultures with 80 parts of soil or more at the end of 11 weeks was very small. It seemed therefore, that 80 parts of this soil was sufficient from which to expect good results.

From preliminary experiments conducted during this time at the Pasteur Institute and from previous experiments with peptone the conclusion was drawn that the activities of the sulfur-oxidizing organisms depended largely

TABLE 7

Influence of ammonium sulfate when the soil is reduced to 10 parts, in a mixture of 100 parts of sulfur and 400 parts of rock phosphate

NUMBER	TREATMENT	INITIAL REACTION		AFTER 11 WEEKS		
				Reaction		Citrate-soluble P_2O_5
		cc.°	*pH*	*cc.*°	*pH*	*per cent*†
1	None. Incubated at 30°C.	0.40	6.8	227.1	3.0	18.72
2	0.2 per cent $(NH_4)_2SO_4$ added at start. Incubated 30°C.	0.48	6.8	242.7	3.0	22.22
3	0.1 per cent $(NH_4)_2SO_4$ added at start. 0.1 per cent added by stirring. Incubated at 30°C.	0.50	6.8	241.3	3.0	21.90
4	None. Inc. at room temperature.	0.40	6.8	175.7	3.0	16.86
5	0.2 per cent $(NH_4)_2SO_4$ added at start. Inc. at room temperature.	0.50	6.8	172.9	3.0	16.18
6	0.1 per cent $(NH_4)_2SO_4$ added at start. 0.1 per cent added by stirring. Inc. at room temperature.	0.50	6.8	183.1	3.0	16.98

° † Same as table 1.

on sufficient available nitrogen. During the progress of the work a more concentrated culture of strong sulfur-oxidizing organisms for inoculation had been secured and was used in the experiments recorded in table 7.

A mixture of 10 parts of soil, 100 parts of sulfur and 400 parts of rock phosphate was used. It had previously been found that the quantity of sulfur could be reduced to 100 parts, as reported in series 5.

The 10 gm. of soil were of a rich garden soil used in the experiments reported in the previous table. In addition, ammonium sulfate equivalent to 0.2 per cent of the mixture, was given to the cultures 2 and 4 at the beginning, and

ammonium sulfate equivalent to 0.1 per cent of the mixture to cultures 3 and 6, while another 0.1 per cent was added to cultures 3 and 6 stirred into during the first 6 weeks.

The available figures show that the compost mixtures to which ammonium sulfate was added and which were incubated at 30°C. made 3.0, 6.0 and 3.2 per cent, respectively, more phosphoric acid available after 11 weeks than the ones with no additions of ammonium sulfate. The mixtures placed at room temperature, however, were all practically alike after this incubation period. It seemed therefore, that enough nitrogenous matter was available up to a certain point. After this point is reached the nitrogen supply seems to be insufficient to keep the organisms at maximum production. Ammonium sulfate furnishes their needed nitrogen. From other experiments (some of which are reported in table 10) it is known that if the quantities of a calcareous soil are reduced to 10 parts or less, sulfur oxidation by the organisms is very slow if not inhibited. It is interesting to note that in the case of these mixtures the influence of temperature was far less pronounced than in the experiments reported in an earlier series. A part of the cultures placed at room temperature were taken away and analyzed at the end of 18 weeks. They had not been stirred during the last 7 weeks and evaporation losses were restored only twice. At the end of this period 27.6 per cent P_2O_5 was available in the mixtures without ammonium sulfate and 34.1 per cent P_2O_5 available in the mixtures to which ammonium sulfate was added.

A part of this relative rapidity of oxidation was attributed to the fact that the small amount of soil did not hinder the production of free sulfuric acid and that the ammonium sulfate added resulted in a slightly acid medium at the beginning of the experiments.

Series 5. Reduction of quantity of sulfur

In the commercial methods of making acid phosphate the proportions of sulfuric acid (52° Bé) and rock phosphate used are approximately equal. The amounts of sulfur employed in composting experiments have usually been larger than the amounts of sulfur used in the commercial methods. If smaller quantities were employed the available phosphoric acid decreased. From a commercial point of view it is interesting to know the results obtained by using these smaller quantities, namely from 20 to 22 per cent of sulfur, especially where the cost of sulfur is high. An excess of sulfur would inhibit composting as might be done in countries where sulfur can be obtained at a comparatively low price. Experiments were made with a soil-rock phosphate mixture in which the amounts of sulfur were reduced to 50 per cent of the original amounts used by McLean and which was pronounced by him as the most economical for farmers. The soil used was a slightly alkaline garden soil containing apparently sufficient nitrogen for the sulfur-oxidizing organisms to produce good results. The compost mixtures were incubated at 30°C. and a part of the results obtained are given in table 8.

From the reported data it is obvious that less sulfur than the theroretical amounts necessary to transform the total phosphoric acid to a soluble form are impractical. It may be seen from the data in this table that a quantity of about the theoretical amount is sufficient to warrant good results. One hundred grams of sulfur, 100 gm. of soil and 400 gm. of rock phosphate gave as good results as 110 gm. of sulfur with the same proportional amounts of soil and rock phosphate, and nearly as good as when 120 gm. of sulfur was employed. When the sulfur was reduced too far the available phosphoric acid decreased accordingly.

TABLE 8

Influence of reducing quantity of sulfur on the production of acidity and available phosphoric acid in composts of 100 parts of soil and 400 parts of rock phosphate incubated at 30°

NUMBER	AMOUNT OF SULFUR USED	INITIAL REACTION		AFTER 18 WEEKS		
				Reaction		Citrate-soluble P_2O_5
	gm.	*cc.°*	*pH*	*cc.°*	*pH*	*per cent†*
1	120	0.4	6.9	266.0	2.9	25.0
2	110	0.4	6.9	247.0	3.0	24.4
3	100	0.4	6.9	247.2	3.0	24.2
4	90	0.4	6.9	246.0	3.0	24.0
5	80	0.4	6.9	213.0	3.0	18.2
6	70	0.4	6.9	204.2	3.0	16.1
7	60	0.4	6.9	201.0	3.1	13.2

° † Same as table 1.

Series 6. Initial reaction

To make a fair test as to whether or not initial reaction would have any influence, 100 parts of an alkaline garden soil, 100 parts of sulfur and 400 parts of rock phosphate were composted and treated at the beginning of the experiment with different amounts of sulfurous acid in some cases, and in other cases sulfurous acid was added to the mixtures when they were stirred for aeration. From the preliminary experiments it was concluded, that approximately 13 cc. sulfurous acid of a strength that will neutralize the same volume of normal sodium hydroxid added per 100 gm. mixture was best suited for the purpose. The mixtures were incubated at room temperature and at 30°C. The data secured are reported in table 9.

The influence of sulfurous acid on the mixtures is very obvious. Although the influence after 15 weeks on the mixtures incubated at 30°C. is not so pronounced as on the mixtures incubated at room temperature, still considerably more phosphoric acid was made available than in the check cultures. The mixtures incubated at room temperature to which sulfurous acid was added at the beginning showed up very favorably in comparison with culture 1 which received no sulfurous acid and was incubated at 30°C. It should be remarked here that a strong culture was used for inoculation similar to that

mentioned under series 4. The addition of sulfurous acid by stirring into the mixtures at the times of aeration proved not to be so effective in each case, showing that possible oxidation of sulfurous acid has but little, if any, effect. Since these studies were mainly concerned with reducing the first part of the incubation period before sufficient free acid or acid salts are produced to made P_2O_5 available, the experiments were not continued.

TABLE 9

Influence of initial reaction, using sulfurous acid, on the production of acidity and available phosphoric acid

NUMBER	TREATMENT PER 100 GM. MIXTURE	INITIAL DETERMINATIONS			AFTER 15 WEEKS		
		Reaction		Citrate-soluble P_2O_5	Reaction		Citrate-soluble P_2O_5
		cc. alk.	*pH*	*per cent*†	*cc.*°	*pH*	*per cent*†
1	None (30°C.)	0.4*	7.3	0.00	211.4	3.0	12.66
2	13 cc. sulfurous acid added by stirring (30°C.)	*acid* 0.4°	6.6	0.00	231.8	3.0	14.12
3	13 cc. sulfurous acid added at start (30°C.)	1.4	6.0	trace	247.6	3.1	17.94
4	None (room temperature)	*alk.* 0.4	7.3	0.00	91.2	3.4	3.08
5	13 cc. sulfurous acid added by stirring (room temperature)	*acid* 0.4	6.6	0.00	194.7	3.1	11.89
6	13 cc. sulfurous acid added at start (room temperature)	1.4	6.0	trace	218.5	3.0	15.38

* † ° Same as table 1.

In a recent publication Lipman and Joffe (9) report that initial reaction is of no advantage when sulfuric acid is used. The mixtures worked with by these investigators had however a relatively high hydrogen-ion concentration at the start, a pH value of 5.4, before additions of sulfuric acid were made. When an alkaline soil is used, the increase of acidity from pH 7.3 to 5.8 requires considerable time, because of the fact that the organisms present have to start with an unfavorable medium. The time required for changing the hydrogen-ion concentration from pH 5.8 to pH 3.2 in such mixtures is usually less than that required for the change from pH 7.3 to 5.8, although the actual acidity produced is much greater in the case of the former. Since these investigators used a decidedly acid medium in their experiments, it is possible that the cause for finding no advantage in lowering the pH values through additions of H_2SO_4, might be found in the initial reaction of the mixtures.

Series 8. Aeration

One of the conclusions drawn by McLean (12) is that the question of aeration should receive foremost consideration. He states that the results obtained would make it appear

that the microörganisms which oxidize sulfur are largely aerobic, and hence require an abundant supply of oxygen. The experiments of Shedd (16) show that the stirring of the mixtures had considerable influence, and he concludes that thorough aeration is one of the conditions which promotes most rapid reaction.

Two series of experiments were conducted for the purpose of testing the influence of aeration. The first series was composed of 80 parts of rich alkaline garden soil, 100 parts of sulfur and 400 parts of rock phosphate. To the mixtures were added different ingredients and the cultures placed in a dark cupboard at room temperature. The cultures were divided into two sets. One set was stirred twice a week by removing the compost from the glasses and mixing thoroughly, while the other set was stirred in the same way at the end of 6 weeks when a sample was taken for analyses. Moisture contents were kept at the optimum by weighing at the end of every week. Although all tumblers were kept covered with glass plates, the amounts of water lost from the stirred cultures were far greater than from the cultures which were not removed from the tumblers. The results obtained after 12 weeks are reported in table 10.

In this series of cultures the same was found as in earlier series regarding the influence of sulfurous acid. Sulfurous acid caused considerable stimulation of bacterial activities in the stirred and unstirred mixtures, resulting in relatively higher acidity and greater availability of P_2O_5 as compared with the checks.

Sodium bicarbonate added in solution to this rich soil compost did not stimulate the activities of the sulfur oxidizing organisms but seemed to retard them. This would indicate that the conclusion drawn for the soil poor in organic material and consequently poor in carbon dioxide production was right. There was present in this soil apparently sufficient CO_2 for the work of the organisms and an addition of a CO_2 source had no influence.

Acetic acid proved to be detrimental in this soil and a combination of 0.02 per cent aluminum sulfate and 0.02 per cent ferrous sulfate had no influence. These two salts had no influence upon the activities of the organisms in any of the calcareous soils used.

All stirred mixtures were considerably in advance as compared with the unstirred mixtures. The conclusion drawn by McLean and Shedd that an abundance of oxygen favored sulfur oxidation seemed right. It was thought therefore, that a still greater abundance of oxygen would help accelerate the reaction still more. An apparatus was made in such a way as to have a small continuous stream of moist air running thru the cultures. A diagram of the apparatus used is given in figure 3.

As a soil compost mixture, 10 parts of a neutral calcareous soil, 100 parts of sulfur, and 400 parts of rock phosphate was used. To replace the bulk of the soil a number of cultures received 0.2 per cent ammonium sulfate. The water-holding capacity of this mixture was 22 per cent.

To some of the cultures 10 cc. sulfurous acid in addition was given, while others received the same amount of sulfurous acid at the time of stirring for aeration. Some of the mixtures through which air was running received a similar amount of sulfurous acid at the beginning and others by means of

TABLE 10

Influence of aeration in the presence of different ingredients on the production of acidity and citrate soluble phosphoric acid

NUMBER	TREATMENT	INITIAL REACTION		AFTER 12 WEEKS		
				Reaction		Citrate soluble P_2O_5
				Stirred twice a week		
		cc.	*pH*	*cc.°*	*pH*	*per cent†*
1	None	alk. 0.18*	7.2	31.3	4.3	2.27
2	14 cc. H_2SO_4	alk. 0.18	7.2	54.5	3.9	5.96
3	13 cc. sulfurous acid	acid 0.46°	6.6	137.0	3.2	10.40
4	0.01 per cent $NaHCO_3$	alk. 0.04	7.1	20.3	5.4	1.51
5	6 cc. 1.0 *N* CH_3COOH	alk. 0.14	7.0	5.7	5.6	0.00
6	0.02 per cent $Al_2(SO_4)_3$ and 0.02 per cent $FeSO_4$	alk. 0.18	7.2	27.4	4.1	1.89
				Stirred once every 6 weeks		
7	None	alk. 0.18	7.2	8.2	5.5	trace
8	14 cc. H_2SO_4	alk. 0.18	7.2	44.4	3.9	4.08
9	13 cc. sulfurous acid	acid 0.46	6.6	71.6	3.5	6.51
10	0.01 per cent $NaHCO_3$	alk. 0.04	7.1	4.7	5.7	0.00
11	6 cc. 1.0 *N* CH_3COOH	alk. 0.14	7.0	1.9	5.9	0.00
12	0.02 per cent $Al_2(SO_4)_3$ and 0.02 per cent $FeSO_4$	alk. 0.18	7.2	9.3	5.4	trace

* † ° Same as table 1.

moist air which went through a wash bottle containing the calculated amounts of weak sulfurous acid per 100 gm. mixture.

The mixtures through which air was running were taken out of the containers and thoroughly mixed at the end of every two weeks. The cultures in the tumblers were stirred once at the end of each week. Preliminary experiments were made to determine the amounts and rate of air flowing through

the mixtures. It was concluded best to use approximately 1 liter of air per hour per 500 gm. of mixture. All mixtures were kept at room temperature. Acidity and hydrogen-ion concentration determinations were made every two weeks, and the soluble phosphoric acid at the end of 14 weeks. The results are recorded in table 11.

While the difference between stirred and unstirred mixtures was considerable, the difference between aerated and stirred composts was still greater. Here, however, the difference was in the opposite direction. All aerated mixtures produced not only less relative acidity, but the available posphoric acid was nil in the aerated cultures (no. 1–4) after 14 weeks. The aerated

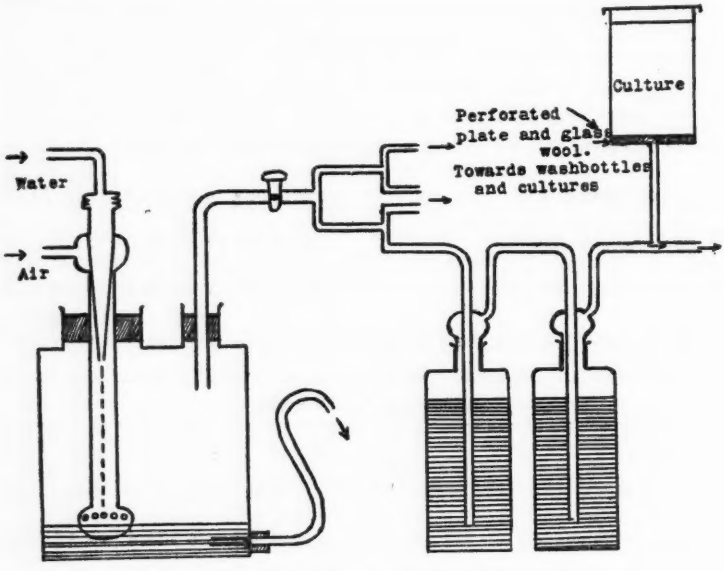

FIG. 3. APPARATUS USED IN AERATION EXPERIMENTS

Washbottles contain water or sulfurous acid. Stream of air is regulated by stopcock

cultures showed some activity after two weeks but this activity decreased as time went on. In the case of number 4 a part of the measured acidity in all probability was free sulfurous acid which went through, as indicated both by titration and hydrogen-ion concentration. Every 4 weeks moisture determinations were made on the mixtures. These determinations showed that the optimum moisture content was very constant.

The stirred mixtures increased regularly in acidity, while the mixtures to which ammonium sulfate was added, in spite of the neutral calcareous soil, produced from the very beginning a much greater acidity than the check cultures without ammonium sulfate. The soluble P_2O_5 in these mixtures at

TABLE 11

Influence of aeration on the change of hydrogen-ion concentration, production of acidity and soluble phosphoric acid

NUMBER	TREATMENT	INITIAL REACTION		AFTER 6 WEEKS		AFTER 10 WEEKS		AFTER 12 WEEKS		AFTER 14 WEEKS		
				Reaction		Reaction		Reaction		Reaction		Soluble P_2O_5
		cc.°	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*per cent*†
1	Stream of moist air	0.4	6.8	0.0	6.9	0.4	6.8	1.1	6.6	4.7	6.3	0.00
2	0.2 per cent $(NH_4)_2SO_4$ stream of moist air	0.4	6.8	11.2	5.1	10.8	5.6	16.5	5.3	9.5	5.5	trace
3	0.2 per cent $(NH_4)_2SO_4$, 10 cc. sulfurous acidaddedat start, stream of moist air	1.6	6.4	11.0	5.1	7.6	5.7	13.3	5.5	5.7	5.9	0.00
4	0.2 per cent $(NH_4)_2SO_4$,10 cc. sulfurousacid in water through which air ran	1.6	6.4	71.0	3.6	58.1	3.7	62.1	4.2	37.0	5.0	trace
5	None	0.4	6.8	1.0	5.7	3.2	5.6	14.6	4.9	18.1	4.8	0.00
6	0.2 per cent $(NH_4)_2SO_4$	0.4	6.8	57.7	3.8	131.7	3.2	154.0	3.3	170.0	3.0	9.66
7	0.2 per cent $(NH_4)_2SO_4$,10 cc. sulfurous acidadded at start	1.6	6.4	57.4	3.7	141.7	3.1	165.9	3.3	180.5	3.0	10.70
8	0.2 per cent $(NH_4)_2SO_4$,10 cc. sulfurous acidadded by stirring	1.6	6.4	59.8	3.8	152.4	3.1	174.2	3.2	190.0	3.1	9.86

° † Same as table 1.

the end of 14 weeks was approximately 10 per cent, all being close together as well in acidity as in available phosphoric acid.

There seems no doubt but that in these experiments too great a quantity of air does not favor sulfur oxidation by the organisms as was supposed.

There seems to be a limit to the quantity of oxygen which acts favorably on the organisms, but where the limit lies could not be determined. The quantities of air which went through, namely 24 liters per 500 gm. per day, were apparently too great, although 200 liters per ton per hour does not seem such an extreme abundance.

Observations

It was noticed throughout a number of series that the relative acidity increased regularly, but the intensity of the acid produced as measured by the

TABLE 12

Relative acidity, pH values and available phosphoric acid in aggregates as compared with the mixed compost

MATERIAL	REACTION		CITRATE SOLUBLE P_2O_5
	cc.°	*pH*	*per cent†*
Coarse aggregates (about 4–5 mm. in diameter)	197.0	3.0	16.48
Medium size aggregates (about 2–3 mm. in diameter)	190.2	3.1	15.92
Small aggregates (about 1 mm. in diameter)	185.6	3.1	12.38
Finely sifted mixture	121.8	3.2	8.86
Unsifted mixture	171.6	3.1	11.42

° † Same as table 1.

hydrogen-ion concentration, varied. The concentrations became higher, till a certain point (3.1 to 2.9) was reached, then became slightly weaker and then back to the same point as before (3.1 to 2.9) to remain there. This point seemed to indicate when the greatest production of available phosphoric acid began. The hydrogen-ion concentration, however, does not necessarily have to be at a certain point, pH 3.2 for instance, to mark the beginning of the availability of phosphoric acid.

The titration measurements of the relative acidity were affected by the soluble phosphates, etc., and they do not, therefore, express the exact amounts of sulfates and free acid formed. The constant action of the free acid on the tri-calcium phosphate may account for the irregularities noticed in the determinations of the hydrogen-ion concentrations. The point at which the greatest amounts of P_2O_5 are made available corresponds with the hydrogen-ion concentration of acid phosphate, which is generally 3.0 to 3.2 as expressed in pH values.

Throughout this work it was freuently noticed that the mixtures had a tendency to form small aggregates composed of soil, rock phosphate and sulfur. Upon examination of these aggregates under the microscope they appeared to have a peculiar structure of indefinite form. A great number of these aggregates were picked out and their relative acidity, hydrogen-ion concentration and available P_2O_5 determined. A part of the mixture as ordinarily taken was used for similar determinations, and another part cautiously sifted. The differences found are tabulated in table 12.

On account of this peculiarity these aggregates or crumbs were later on broken up when the composts were mixed and when samples were taken for analyses.

Often it was found that the incubated mixtures produced hydrogen sulfide during the first weeks of the incubation period. Sometimes it was only noticeable by the smell and at other times even by a black color produced. Small additions of sulfuric acid favored the sulfur-oxidizing organisms and depressed the hydrogen sulfide-producing organisms. Such cultures were always excluded in the final results and are not included in the tables.

CONCLUSIONS

1. Sulfur oxidation takes place in a calcareous slightly alkaline soil and does not hinder the solubility of phosphoric acid.

2. Compost mixtures incubated at 30°C. increased more rapidly in relative acidity and available P_2O_5 than composts kept at room temperature. When larger quantities of a calcareous soil is used the influence of temperature is usually greater than when small amounts of such a soil are employed.

3. The influence of light is slightly detrimental to sulfur oxidizing-organisms.

4*a*. Small quantities of sulfuric acid stimulate bacterial activities particularly during the first weeks of the incubation period, if calcareous, slightly alkaline soil is used.

b. Sodium bicarbonate stimulated the bacterial activities considerably when a calcareous soil poor in organic material was used; if a similar soil rich in organic material was used it failed to produce stimulation to the same extent, presumably due to greater CO_2 production in this soil.

c. Sodium bisulfate in solution and acetic acid had no stimulating effect, but rather detrimental.

d. A mixture of 0.02 per cent ferrous sulfate and 0.02 per cent aluminum sulfate failed to exert any influence.

5. Partial sterilization of the soil and the mixtures by additions of sodium chloride and by hydrochloric acid proved of no value. NaCl retarded the activities of the sulfur-oxidizing organisms, and the same was true to a less extent with HCl.

6*a*. The amounts of soil can be reduced with success from 16 to 17 per cent to 1.6 to 1.7 per cent of the mixtures.

b. It is possible to replace the bulk of a calcareous soil poor in nitrogenous material with ammonium sulfate.

7. The quantities of sulfur in the mixtures, reduced to approximately the amounts required in ordinary factory methods of making acid phosphate, gave good results.

8. Addition of small amounts of sulfurous acid to change the initial reaction of these mixtures in which a slightly alkaline calcareous soil was used, proved to have a decided influence upon the rapidity of accumulation of acidity and available phosphoric acid.

9. Aeration of the mixtures had considerable influence, but when the mixtures received an abundance of air, sulfur oxidation nearly ceased and no phosphoric acid was made available.

10. The hydrogen-ion concentration in the mixtures changed till pH values of from 3.1 to 2.9 were reached, indicating the point at which P_2O_5 becomes most rapidly available. Relative acidity, as measured by the titration method, accumulated after this point was reached.

11. The mixtures have a tendency to form aggregates which have a different relative acidity and different quantities of available P_2O_5 according to the size of the crumbs.

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THE INFLUENCE OF SOIL REACTION UPON THE GROWTH OF ACTINOMYCETES CAUSING POTATO SCAB[1]

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The close correlation between soil reaction and the growth of specific microörganisms in the soil has recently been made the subject of investigations from various viewpoints. By expressing the soil reaction in terms of hydrogen-ion concentrations, we are employing a factor which has greater significance in biochemical soil processes than merely the amount of acid substance present. In studying the relation between the hydrogen-ion concentration of the soil and the activities of microörganisms, we must differentiate between the limiting reaction, at which beneficial microörganisms become active in the soil, from that reaction at which microörganisms injurious in one way or another to the soil microflora or the growth of higher plants are checked in their development. The work of Gainey (7) and others takes up the influence of reactions upon the growth of *Azotobacter*, Fred and associates (6) and others on *B. radicicola*, Meyerhof (24) on Nitrosomonas and Nitrobacter. The limitation of the growth of actinomycetes causing potato scab can serve as an excellent illustration of the second group of phenomena. However, when we attempt to adjust the reaction of the soil to a certain hydrogen-ion concentration with a view of limiting the growth of an injurious organism, we ought not omit from consideration the influence that this reaction may exert upon the growth of beneficial organisms.

The organism causing potato scab was first described by Thaxter (28, 29) under the name *Oospora scabies*. In a study of "scab" of sugar beets, Krüger (17) suggested that Oospora can probably be classified with the streptothrices (actinomycetes). It was later identified by Cunningham (5) with *Actinomyces chromogenus* of Gasperini. However, the term *A. chromogenus* based upon the fact that the organism produces a dark brown pigment on organic media is hardly appropriate, since, as pointed out by Krainsky (16), Waksman (31, 32), and Conn (4), there is a large number of actinomycetes in the soil, which are able to produce this pigment. This factor was readily recognized by Güssow (12), who very appropriately suggested the term *Actinomyces scabies*, which should be used in the interests of an accurate nomenclature. Even with a correct nomenclature, we must be on the lookout for other actinomycetes not of the *A. scabies* type. From 15 to 40 per cent of the soil's microbial flora consists of actinomycetes and one may readily isolate from a diseased potato tuber another Actinomyces which will not be "scabies" at all and which may differ much in its

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metabolism, including the limiting reaction for growth. Only recently, Wollenweber (38) pointed out the potato scab is caused not by one species of Actinomyces, but by a number of them. At least 6–7 Actinomyces species have been isolated from diseased potatoes and from the soil, and their pathogenicity demonstrated by inoculation tests. As a result of these studies of Wollenweber and of the writer, which will be published at a later date, we may have to limit the term *A. scabies* (Thaxter) Güssow to one specific type, from which other actinomycetes that are able to cause potato scab may be differentiated. However, for the present, we may speak of the organism causing common potato scab both in America and in Europe (as pointed out by Wollenweber), as the *Actinomyces scabies* group.

One of the most important and most promising methods of controlling the development of potato scab in the field is the use of acid forming substances. Fertilizers which leave the reaction of the soil alkaline have been looked upon as injurious, while those which leave the soil acid, like ammonium sulfate and acid phosphate, are generally preferred. The use of sulfur for combating scab has been recognized by Halsted (13, 14). But the results obtained by the various investigators are lacking in uniformity. Even Halsted (15) found that an application of 300 pounds of sulfur per acre was effective, within one year under certain conditions, while an application of 600 pounds of sulfur in 1896 was ineffective, but became effective in 1898.

These discrepancies were due to the fact that the basic underlying principles were not recognized, namely that the sulfur is oxidized in the soil by certain microörganisms to sulfuric acid, and the increase in soil acidity leads to a decrease in scab. Two factors are to be considered here; the presence of the proper sulfur-oxidizing organisms in the soil and the reaction and character of the soil itself. The amount of sulfur to be used will depend upon the latter factor.

This lack or correlation between the application of sulfur and control of scab is also true of the other experiments where the addition of sulfur failed to diminish the amount of scab as those of Garman (8) and Brooks (1). However, where enough sulfur was used year after year and the soil conditions were proper, a definite reduction of the potato scab was effected by Wheeler and Adams (36), Sherbakoff (26, 27) and Lint (18, 19). Even in as late a publication as that of Wollenweber (38) in 1920, where the importance of making the soil acid for the control of potato scab has been well recognized, the application of sulfur was not found to give uniform results.

Only with the development of methods for determining the correct reaction of the soil as expressed in terms of hydrogen-ion concentration, and with an increased knowledge of the microörganisms concerned in the oxidation of sulfur in the soil, could we understand the underlying principles and place our knowledge of scab control on a systematic foundation. A third factor should be considered here which is not of least importance, and that is the metabolism of the causative agent of potato scab, *A. scabies*. Our information on the soil reaction has been advanced by the researches of Sörensen and Michaelis in Europe, Clark and Lubs and others and particularly that of Gillespie (10, 11) in this country, as applied to scab control. Our knowledge of the mechanism of sulfur oxidation in the soil by crude cultures of microörganisms has been advanced by the work of Lipman and his associates (20, 23) and McLean (24), and with pure cultures by Waksman and Joffe (33, 34, 35). As to the metabolism of *Actinomyces scabies* itself, it has been taken up by Lutman and Cunningham (21) and by Waksman (30).

Considering the practical application of the subject, the following questions are important:

1. Is there a limiting acid reaction for the species of *Actinomyces scabies* which wlll absolutely prevent their development?
2. By what certain means can we obtain this reaction?

3. What is the influence of type of soil, amount of organic matter in the soil, moisture content, aeration, etc.?

These three points will be taken up in order and experimental results presented.

LIMITATION OF GROWTH OF A. SCABIES BY ACIDITY OF MEDIUM OR SOIL

Gillespie and Hurst (10) were the first to call attention to a characteristic difference of hydrogen-ion concentration shown by the extracts of soils of the types Caribou loam and Washburn loam. These types, similar in texture and derived from the same glacial till, differ, however, due to local topographic differences. In reaction, the Caribou loam is more intensely acid than the Washburn, with a relative freedom from scab of potatoes grown in the more acid soil. Gillespie and Hurst (11) have further shown that soils having a hydrogen-ion concentration equivalent to pH 5.2 rarely produced scabby potatoes, while less acid soils generally produced scabby potatoes, unless it was new land. In another paper, Gillespie (9) found that, by growing the organisms causing potato scab on two synthetic media and upon a medium prepared from potato extract at reactions of pH = 5.2 and less, the growth was much slower and generally less vigorous than in less acid media. It is interesting to note that Gillespie (9) observed a marked decrease in acidity accompanying the growth of the organisms. This change in reaction of media was pointed out by Waksman (31) for the Actinomyces group as a whole. Wollenweber (38) stated that actinomycetes causing potato scab are very sensitive to acid (0.05 per cent) and mu h less sensitive to alkali (0.25 per cent).

EXPERIMENTAL

The influence of reaction upon the growth of actinomycetes, in general, and species causing potato scab, in particular, was studied both in solution and in soil. The cultures used were obtained from Dr. Morse of the Maine Agricultural Experiment Station and Mr. M. Shapovalov of the U. S. Department of Agriculture except for three which were isolated by Waksman and Curtis (32).

The following cultures were used in one or another of the experiments:

A. scabies 222 and 254 were obtained from Dr. Morse, who isolated them from potatoes in Maine in 1906 and in 1914 respectively.

A. scabies 259 was obtained from Dr. Morse, who isolated if from potatoes coming from Ohio in 1915.

A. scabies 281, 283 and 295 were obtained from Mr. Shapovalov, who isolated them from potatoes infected with common scab in Maine.

A. griseus was isolated by the writer and Curtis (32) from the soil in 1916. This culture has a strong proteolytic power (30).

A. violaceus-ruber and *A. viridochromogenus* were also isolated in 1916 by the writer and Curtis from the soil and described in detail elsewhere (31). These two cultures are quite similar, in description, to Wollenweber's *A. tricolor* and *A. aerugineus*.

The organisms were grown on synthetic agar, Czapek's or dextrose agar (31), and were then inoculated into the sterile liquid medium. For the inoculation of sterile soil, the cultures were grown on the liquid synthetic media, in 250-cc. Erlenmeyer flasks and, for inoculation, 1 cc. of the culture, well shaken previously, was introduced, under sterile conditions, into each flask.

Experiment 1

The medium used in the first experiment had the following composition:

	grams
Glycerin	20.0
Asparagin	5.0
$MgSO_4$	0.5
KCl	0.5
$FeSO_4$	0.01

Phosphates in varying amounts. Distilled water to make 1000 cc. (after adding the buffers).

Phosphoric acid, KH_2PO_4, K_2HPO_4 and NaOH were used to adjust the reaction of the medium to the desired hydrogen-ion concentration, as shown in table 1.

TABLE 1

The amounts of buffer solutions used in experiment 1 and the hydrogen-ion concentration of the media

MEDIUM NUMBER	BUFFER SOLUTIONS				REACTION
	H_3PO_4 *1.0 N*	KH_2PO_4 *1.0 M*	K_2HPO_4 *1.0 M*	NaOH *1.0 N*	
	cc.	*cc.*	*cc.*	*cc.*	*pH*
1	4	16	....	....	3.2
2	2	18	....	....	3.6
3	....	20	....	....	4.2
4	....	19.5	0.5	....	4.6
5	....	19.0	1.0	....	5.2
6	....	18.0	2.0	....	5.6
7	....	16.0	4.0	....	6.4
8	....	4.0	16.0	....	7.0
9	....	....	20.0	....	7.5
10	....	....	16.0	4	8.2

In all media, a total of 20 cc. of the buffer solutions were added per liter. The media were distributed, in 100-cc. portions into 250-cc. Erlenmeyer flasks and sterilized at 15 pounds pressure for 15 minutes. The flasks were then inoculated from slants with a culture 7–14 days old by means of a platinum loop. They were incubated at 25–27°C. for 21 days and filtered through weighed filter paper. The growth on the filter paper was dried at 80°C. to constant weight. Under "growth", the relative amount of growth is given, while under "weight" actual weight of the mycelium is given, in milligrams. The pH values were obtained by the use of the indicator method, as outlined by Clark and Lubs (2). Results are presented in table 2.

The limiting acid reaction for the two cultures of *A. scabies* seems to be at a pH between 4.6 and 5.0, while the limiting acid reaction for *A. griseus* and *A. violaceus ruber* isolated from the soil lies between pH 4.2 and 4.6. This indicates a greater acid resistance for the saprophytic forms. As to the

alkaline limit, *A. scabies* 259 did not develop above pH 7.4. When good growth took place, the reaction of the medium was invariably changed to alkaline. This is due to the fact that the actinomycetes as a group, with perhaps few exceptions, do not seem to form any acid from the carbon sources, as in the case of many bacteria and fungi, but produce alkaline substances from the nitrogen sources [Waksman (30)]. In this case, it is the production of ammonia from the asparagin which makes the medium alkaline. By determining the ammonia formed, we can account almost quantitatively for the change in reaction.

TABLE 2

Growth of actinomyceles in synthetic media at various hydrogen-ion concentrations and changes of the reaction produced.

MEDIUM	REACTION OF CONTROL	A. SCABIES 259			A. SCABIES 222			A. GRISEUS			A. VIOLACEUS RUBER		
		Relative growth*	Wt.	Final reaction	Relative growth	Wt.	Final reaction	Relative growth	Wt.	Final reaction	Relative growth	Wt.	Final reaction
	pH		*mgm.*	*pH*		*mgm.*	*pH*		*mgm.*	*pH*		*mgm.*	*pH*
1	3.4	0	0	3.4	0	0	3.4	0	0	3.4	0	0	3.4
2	3.8	0	0	3.8	0	0	3.8	0	0	3.8	0	0	3.8
3	4.2	0	0	4.2	0	0	4.2	0	0	4.2	0	0	4.2
4	4.6	0	0	4.6	0	0	4.6	T	1†	4.6	T	2†	4.6
5	5.0	T	1	5.0	2	268	5.0	T	1†	5.0	1	15	5.0
6	5.6	T	1	5.6	3	346	5.8	T	1†	5.6	2	47	5.8
7	6.4	1	2	6.4	4	377	6.8	2	214	6.4	3	184	6.6
8	6.9	2	5	7.1	5	601	8.4	3	253	8.0	4	314	7.4
9	7.4	1	2	7.4	5	499	8.6	2	177	8.2	3	233	7.6
10	8.0	0	0	8.0	5	547	9.2	2	110	9.0	1	99	8.0

* T = Trace, 1–5 indicates the relative amounts of growth, 5 being the maximum.
† Approximate weight.

Experiment 2

The medium used in this experiment had the same composition as that used in the previous one, only the amount of asparagin was reduced to 2 gm. per liter of medium and the amounts of buffers in the media were slightly changed, as shown in table 3. The cultures were grown for 21 days at 25–27°C. The results are given in table 4.

We find a decided difference between the various cultures of *A. scabies* in their resistance to acidity. While *A. scabies* 254 did not begin to develop till the pH of the medium was 5.3, *A. scabies* 281 made some growth even at 4.6.

TABLE 3

The amounts of buffer solutions used in experiment 2

MEDIUM NUMBER	BUFFER SOLUTION		REACTION OF MEDIUM
	KH_2PO_4 *1.0 M*	K_2HPO_4 *1.0 M*	
	cc.	*cc.*	*pH*
1	20.0	0.0	4.2
2	19.5	0.5	4.6
3	19.0	1.0	5.0
4	18.5	1.5	5.4
5	18.0	2.0	5.6
6	16.0	4.0	6.2
7	10.0	10.0	6.8

TABLE 4

Growth of A. scabies in synthetic media at various hydrogen-ion concentrations

MEDIUM NUMBER	REACTION OF CONTROL	A. SCABIES 254		A. SCABIES 259		A. SCABIES 281	
		Relative growth	Final reaction	Relative growth	Final reaction	Relative growth	Final reaction
	pH		*pH*		*pH*		*pH*
1	4.0	0	4.0	0	4.0	0	4.0
2	4.6	0	4.6	0	4.6	1	5.2
3	4.9	0	5.0	1	6.6	2	5.8
4	5.3	2	5.4	2	6.1	3	6.2
5	5.6	3	6.8	2	6.2	3	6.6
6	6.1	3	6.6	3	7.4	....	....
7	6.8	....	....	3	7.4	....	....

Experiment 3

The above experiment was repeated with larger amounts of buffer (50 cc. of the mixture) and 2 gm. of asparagin per liter. The flasks contained only 60 cc. of culture medium. Five cultures of *A. scabies* were used, with three or four flasks for each culture and each reaction. The amount of buffer used in experiment 3 is given in table 5 and the results are presented in table 6.

TABLE 5

The amount of buffer solutions used in experiment 3

MEDIUM NUMBER	KH_2PO_4 *1.0 M*	K_2HPO_4 *1.0 M*	REACTION OF MEDIUM
	cc.	*cc.*	*pH*
1	50.00	0.00	4.2
2	48.75	1.25	4.8
3	47.00	3.00	5.2
4	45.00	5.00	5.6
5	40.00	10.00	6.4

From the results presented in table 6, we may definitely establish the fact that, when the nutrients present in the medium are favorable for the development of actinomycetes, the limiting acid reaction, for the majority of strains, lies at a pH between 4.8 and 5.2. While some strains (281) may develop to

TABLE 6

Growth of A. scabies in synthetic media at various hydrogen-ion concentrations

INCUBATION PERIOD	REACTION OF MEDIUM	A. SCABIES 222			A. SCABIES 254			A. SCABIES 281			A. SCABIES 283		A. SCABIES 295	
		gr.*	wt.	pH	gr.	wt.	pH	gr.	wt.	pH	gr.	pH	gr.	pH
days	*pH*		*mgm.*			*mgm.*			*mgm.*					
	4.2	0			0			0			0		0	
	4.8	0			0			0			0		0	
5	5.2	1			0			0			1		0	
	5.6	2			T			0			2		0	
	6.4	3			2			1			3		1	
	4.2	0			0			0			0		0	
	4.8	0			0			0			0		0	
10	5.2	2			1			1			2		0	
	5.6	3			2			2			3		T	
	6.4	4			3			3			5		2	
	4.2	0			0			0			0		0	
	4.8	0			0			T			0		0	
15	5.2	3			1			2			2		0	
	5.6	4			2			3			4		1	
	6.4	5			3			4			5		2	
	4.2	0		4.2	0		4.2	0		4.2	0	4.2	0	4.2
	4.8	0		4.8	0		4.8	T	4	5.2	0	4.8	0	4.8
20	5.2	3	80	5.4	1	10	5.4	2	18	5.8	2	5.5	0	5.2
	5.6	5	190	6.5	3	60	6.3	4	42	6.7	4	6.2	1	5.9
	6.4	5	225	6.9	3	48	6.8	4	120	7.1	5	7.0	2	6.6
	4.2	0		4.2	0		4.2	0		4.2	0	4.2	0	4.2
	4.8	0		4.8	0		4.8	2	30	6.3	T	5.0	0	4.8
30	5.2	3	70	5.4	2	40	5.8	3	60	7.3	3	5.4	0	5.2
	5.6	5	200	7.1	4	120	6.6	3	65	6.8	5	6.4	1	6.0
	6.4	5	250	7.3	4	125	6.8	4	100	7.4	5	7.0	2	6.6

* gr. = relative growth; wt. = actual weight of mycelium; pH = pH value of culture at the end of the particular period.

a slight extent at 4.8 and, given a long enough period of incubation may even make a fair growth at that reaction, others (295) will begin to develop only at a much higher point, namely at about pH 5.6. It is quite possible that in the first case, the organism is able to change rapidly the reaction of the medium to less acid and make conditions more readily favorable for its de-

velopment than do the other strains. Culture 295 made a rather weak growth even under optimum conditions; this is due probably to the fact that this strain has lost its power of producing aerial mycelium, perhaps on account of long cultivation on artificial media. When a piece of substratum growth is inoculated into a flask with medium, conditions have to be very favorable for its development, before growth can take place. Aerial spores, however, soon germinate and develop into a mycelium, if conditions are only favorable. This is the reason why so many variable results are obtained with various cultures. It is due more to the length of time the organism has been kept on artificial culture media than to a difference in strain. Wollenweber (38) has obtained such a culture from Amsterdam, this culture coming originally from Dr. Whetzel's laboratory at Cornell University. Unable to obtain any scab on potatoes by the use of this culture, Wollenweber merely suggested that it is possible that the American strain does not cause potato scab in Germany.

The change in reaction of medium to less acid confirms the previous observations.

Experiment 4

In addition to the study of the growth of actinomycetes in solution, a series of experiments were carried out, in which actinomycetes, chiefly *A. scabies*, were grown in sterile soil previously adjusted to various reactions, with or without the addition of organic matter. A greenhouse soil and a Sassafras loam were used for this work. A quantity of the two soils were air dried, sieved and brought into the laboratory. The total moisture holding capacity of these soils was 60 and 30 per cent respectively. During the experiment the soils were kept at 60 to 70 per cent saturation unless otherwise stated. The soils, with or without the organic matter, were placed, in 100-gm. portions, in 250-cc. Erlenmeyer flasks, moisture was added, and flasks were plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 1 hour. The flasks were then inoculated with 1-cc. portions of well shaken liquid cultures of the proper organisms, 7 to 14 days old, and incubated at 25 to 28° for the desired length of time. Sterile water was added, at definite intervals, to keep the moisture at an optimum. At the end of the incubation period, some of the soil was used for determinations of ammonia. The method of Gillespie (10) for obtaining the soil solution combined with the colorimetric method for the determination of reaction were used. Ammonia was determined by distillation with MgO into standard acid solution. In some cases the Folin aeration method was used; the results checked up fairly well with those obtained by the distillation method, but were somewhat lower. Since the ammonia formation is given only as an indication of the growth of the organisms and is not an absolute value only the results obtained from the distillation with MgO are reported.

In the first experiment, the greenhouse soil was used; the reaction was adjusted to various pH values by means of sulfuric acid and CaO. Ten-gram portions of soil were placed in beakers, and various amounts of acid and CaO added. To each soil 3 cc. of water was added (the acid was added in the water to make 3 cc.), the beakers covered and allowed to stand 4 to 7 days, when the pH values were determined. Titration curves for the two soils are given in figure 1.

Ten points were selected on the curve, so as to give a series of values, ranging from pH 3.0 to pH 9.8. The proper amounts of acid and CaO were then added to 1-kgm. portions of the soil, the optimum amount of water was then

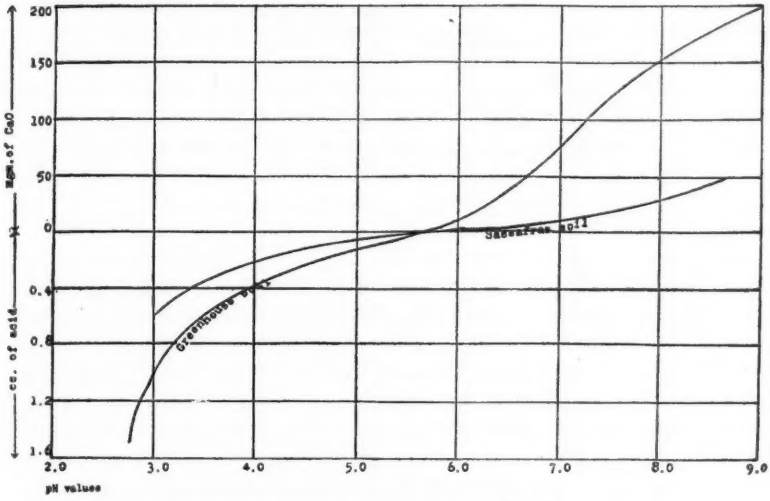

FIG. 1. TITRATION CURVES FOR THE TWO SOILS

The hydrogen-ion exponent (pH value) are given as ordinates and the amounts of 2 *N* acid, in cubic centimeters, or powdered CaO, in milligrams, added to 10-gm. portions of soil, as abscissae.

introduced and the soils allowed to stand in jars till air-dry. The soils were then placed in 100-gm. portions, in flasks, 2 gm. of alfalfa meal or 1 gm. of dried blood were then added, the soils were brought to optimum moisture, sterilized as usual, inoculated with *A. scabies* 222 and the three soil Actinomyces and incubated for 28 days. The flasks were well shaken at the end of 7 days, so as to make the inoculation more uniform. The results are given in table 7.

The sterilization influenced to some extent the reaction of the soil, particularly in the case of the soils made alkaline. In these the reaction became less alkaline, probably because of the absorption of the calcium. The limiting acid reaction is found to lie at about pH 5.0 to 5.2. While *A. scabies* produced,

TABLE 7

The influence of reaction upon the growth of actinomycetes in greenhouse soil

ORIGINAL REACTION OF MEDIUM	CONTROL			A. SCABIES 222						A. VIRIDOCHROMOGENUS						A. GRISEUS					
	Reaction			Afalfa		Dried blood				Alfalfa		Dried blood				Alfalfa		Dried blood			
								NH_3						NH_3						NH_3	
	Alfalfa	Dried blood	NH_3 in 100 gm. soil and dried blood	Gr.*	Final reaction	Gr.	Final reaction	In 100 gm. soil	Increase over control	Gr.	Final reaction	Gr.	Final reaction	In 100 gm. soil	Increase over control	Gr.	Final reaction	Gr.	Final reaction	In 100 gm. soil	Increase over control
pH	*pH*	*pH*	*mgm.*		*pH*		*pH*	*mgm.*			*pH*		*pH*				*pH*		*pH*		
3.0	3.2	3.2	9.6	0	3.2	0	3.2	8.7	− 0.9	0	3.2	0	3.2	9.1	−0.5	0	3.2	0	3.2	9.3	− 0.3
3.6	3.6	3.6	10.7	0	3.6	0	3.6	9.8	− 0.7	0	3.6	0	3.6	13.7	3.1	0	3.6	0	3.6	11.6	1.0
4.2	4.1	4.0	10.7	0	4.2	0	4.5	12.9	2.2	0	4.2	0	4.4	11.6	0.9	0	4.4	0	4.3	11.8	1.1
5.2	5.0	5.0	9.9	T	5.2	2	6.0	38.8	28.9	T	5.2	2	6.0	19.3	9.4	T	5.2	1	6.2	11.2	1.3
5.8	5.8	5.8	9.1	2	6.0	3	7.3	77.4	68.3	2	6.0	3	6.6	56.6	47.5	1	6.2	2	7.0	50.0	40.9
6.6	6.4	6.4	5.8	3	6.6	3	8.0	70.1	64.3	3	6.6	4	8.0	71.0	65.2	3	7.3	3	8.0	65.8	60.0
7.0	6.8	7.2	5.2	3	7.6	3	8.1	72.1	66.9	4	7.7	3	8.4	68.5	63.3	2	7.8	3	8.4	67.2	62.0
7.7	7.7	7.7	4.6	2	8.0	3	8.4	67.2	62.6	3	8.0	2	8.4	68.2	63.6	2	8.3	3	8.6	57.6	53.0
9.4	8.8	8.8	3.2	T	9.0	T	9.4	3.6	0.4	1	9.0	0	8.8			1	8.6	2	8.8	6.0	2.8
9.8	9.4	9.6	2.4	0	9.6	0	9.6	2.1	− 0.3	0	9.6	0	9.6	2.4	0	0	9.8	0	9.8	2.8	0.4

* Gr. = relative growth.

at this reaction, only a trace of growth with the alfalfa meal, it made a fair growth with the dried blood. This is no doubt due to the rapid decomposition of the latter, with the formation of ammonia which accounts for the change in reaction. As much as fifty per cent of the nitrogen of the dried blood has been liberated in 28 days in the form of ammonia by various actinomycetes when the initial reaction was at an optimum. Although the figures given under growth are only relative, the amounts of ammonia formed are good indications of actual growth that has taken place. Under these conditions, we find that the optimum reaction for both *A. scabies* and the saprophytic actinomycetes is at a pH 5.8 to 7.7, while the limiting reactions are pH 5.0, on the acid side, and pH 8.8, on the alkaline. Above and below these two points no growth took place.

The alkaline limit is so high that it would be impossible to obtain it for any practical purposes, without ruining the fertility of the soil and is comparable only to black alkali soils. The acid limit is that found by Gillespie and Hurst (10, 11) for the potato soils of Maine which give good crops of potatoes practically free from scab. The application of this limit is not only possible, but practical and of immense economic importance.

Experiment 5

To eliminate the rapid change in reaction, due to the presence of easily decomposable organic matter, the experiment 4 was repeated with sassafras soil to which no organic matter had been added. Five strains of *A. scabies* were used. Since the amounts of ammonia formed from the soil organic matter were rather small, the actual numbers of actinomycetes were determined in the flask by the plate method. Of course, we do not get here absolute figures, since no differentiation is made between actinomycetes spores and mycelium. The results are, however, striking even with the relative figures. The method of plating out is similar to that used in the routine soil bacteriological counts, modified egg albumen agar being used for preparing the plates. The details of the method are found elsewhere (31). The results are reported in table 8.

The differences found in the liquid cultures are also observed on sterile soil. *A. scabies* 281 makes a fair growth at pH 5.1, while 295 begins to develop only at pH 5.3. The rapidity of formation of aerial mycelium accounts for the difference in numbers. Cultures 222 and 283 readily form an abundant aerial mycelium and gave, therefore, a count of 80 millions per gram, with an optimum reaction. Culture 295, which formed no aerial mycelium in pure culture, grew much more slowly and gave a maximum of only 1,500,000 per gram. The limiting reaction for the majority of the strains is found at pH 5.0. Culture 281, however, grew at pH 4.8 and others developed only when the reaction was distinctly less acid, pH 5.3 to 5.5. The difference in the strains may also account for some of the discrepancies in scab control by making the soil acid.

TABLE 8

The influence of reaction upon the growth of A. scabies in the soil

ORIGINAL REACTION OF MEDIUM	A. SCABIES 222			A. SCABIES 254			A. SCABIES 281			A. SCABIES 283			A. SCABIES 295		
	Gr.*	Numbers†	Final reaction	Gr.	Numbers	Final reaction	Gr.	Numbers	Final reaction	Gr.	Numbers	Final reaction	Gr.	Numbers	Final reaction
pH			*pH*			*pH*			*pH*			*pH*			*pH*
4.8	0	15,000	4.8	0	8,000	4.8	0	7,000	4.9	0	110,000	4.9	0	3,000	4.9
5.1	1	800,000	5.3	0	9,000	5.1	1	48,000	5.4	?	330,000	5.2	0	20,000	5.1
5.3	2	1,700,000	5.6	1	500,000	5.4	2	500,000	5.6	1	22,000,000	5.5	T	36,000	5.3
5.6	3	51,000,000	5.8	3	1,000,000	6.0	3	1,700,000	5.6	3	40,000,000	5.7	1	250,000	5.5
6.2	4	80,000,000	6.2	3	1,500,000	6.3	3	2,500,000	6.2	3	47,000,000	6.4	2	300,000	6.0
7.2	4	85,000,000	7.4	4	5,000,000	7.6	3	10,300,000	7.7	4	80,000,000	7.5	2	1,500,000	7.6

* gr. = relative amount of growth.

† Organisms per gram of soil.

THE REDUCTION OF SOIL REACTION BY THE UTILIZATION OF THE OXIDATION OF SULFUR BY MICROÖRGANISMS

The use of acidic fertilizers, or such that tend to make the soil reaction more acid has been recommended. These may do for soils that are acid to start with and where the amount of scab may be small. In this case the use of green manures may suffice to control scab, because organic acids formed in the process of the decomposition of the organic matter may be sufficient to exert a temporary check upon the development of scab. However, this acidity is not of a lasting nature, since the organic acids are rapidly broken down in the soil.

To be able to control scab in slightly alkaline, neutral or only slightly acid soils, acid has to be added to the soil so as to make the reaction acid enough to prevent growth of *A. scabies*. The direct use of acid is both unpractical in method of application and injurious in its action upon the beneficial microorganisms. A substance has to be added which gradually becomes acid. Such a substance is sulfur, which will be oxidized to sulfuric acid, provided the proper sulfur oxidizing organisms are present.

A brief review of the literature on the use of sulfur for the control of potato scab has been given above. We might cite here two typical experiments which have a direct bearing upon the problem at hand.

Wheeler, Hartwell and Moore (37) found that by applying sulfur at the rate of 600 pounds per acre in 1896, no benefit was obtained in limiting scab, in several instances, although it seemed to have been of some value in other cases. When 300 pounds of sulfur per acre were applied again to the same soil in 1897, the number of scabbed tubers was reduced 9 per cent and the number of badly scabbed ones, 31 per cent. They suggested then, quite correctly, that sulfur is oxidized in the soil to sulfuric acid and the germicidal action of the sulfur was probably caused by this acid; therefore, a given amount of sulfur would be expected to exert a more marked influence as a preventative of scab on a netural or slightly alkaline soil than on one which was very alkaline or which contained such large quantities of carbonates as not to be materially affected by the limited amount of acid produced. However, they pointed out that the application of sulfur to soils, for preventing potato scab regardless of the character of the reaction of the soil is liable to occasionally cause much injury.

By applying sulfur to soils and studying the resulting reaction Martin (22) found that an increase in soil acidity resulting from oxidation of sulfur led to a marked decrease in the amount of scabby potatoes. Where the initial hydrogen-ion concentration of the soil before the sulfur application was pH 5.8 or less, the lighter applications 300 to 500 pounds of sulfur gave approximately as good control of scab as the heavier applications (700 to 1200 pounds); where the initial reaction of the soil was greater than pH 6.0, the heavier applications of sulfur gave the best control of scab.

Experiment 6

In a study of the influence of oxidation of sulfur of the soil upon the activities of the various groups of soil microörganisms, it was found that small additions of sulfur, as long as the reaction does not become too acid (not below pH 5.0), stimulate the growth of microörganisms as indicated by numbers and the decomposition of organic matter. Of course such microörganisms,

like the nitrifying and nitrogen fixing bacteria, which are active at an optimum reaction above pH 6.0 and more, may be slightly injured in their activities (this has not been demonstrated yet). But as soon as the reaction becomes acid enough to bring down the pH value to less than 5.0 or 5.2, the relative number of actinomycetes to the total bacterial numbers rapidly decreases, as indicated by the results in table 9, which represents a typical instance.

TABLE 9

The influence of sulfur oxidation upon the relative numbers of bacteria and actinomycetes in the soil

SULFUR APPLIED PER ACRE	REACTION	MICROÖRGANISMS PER GRAM	ACTINOMYCETES	
			Numbers per gram	Per cent of total numbers
lbs.	*pH*			
None	6.2	13,600,000	6,100,000	44.8
300	5.6	12,600,000	6,500,000	51.6
600	5.1	4,800,000	1,200,000	25.0
900	4.8	4,000,000	900,000	22.5

Experiment 7

Several experiments were carried on for the purpose of demonstrating the fact that the oxidation of sulfur by a pure culture of *Thiobacillus thiooxidans* will result in preventing or limiting the growth of pure cultures of *A. scabies* in the same soil. But experiments of this nature are rather difficult to carry out. When sulfur and *Th. thiooxidans* are added first to the soil, and the *A. scabies* is introduced only after the oxidation of the sulfur has gone so far as to make the reaction of the soil acid enough, no growth of *A. scabies* will take place. This treatment is equivalent to making the soil acid by means of sulfuric acid. When *A. scabies* is introduced in the beginning of the experiment, it will make the reaction of the soil alkaline enough, particularly in the presence of a protein-rich substance like dried blood, to neutralize the acidity formed from the oxidation of sulfur. This is particularly true of sterile soil, where the actinomycetes begin to develop rather rapidly while the action of *Th. thiooxidans* is slow at first. These experiments are therefore not representative of what actually takes place in normal soil.

An experiment will therefore be reported, in which the greenhouse soil was used, both with and without the addition of 1 per cent dried blood. Where sulfur was used (100–150 mgm.), it was added to 100-gm. portions of the soil, before sterilization, which took place as usual. The relative amount of growth, ammonia content, and the numbers of actinomycetes per gram of soil are reported as an index of the activities of *A. scabies*. The flasks were inoculated with 1-cc. portions of a 14-day old liquid culture of *Th. thiooxidans* and *A. scabies* strains.

Whenever sulfur was used in connection with *Thiobacillus thiooxidans*, there was found a limitation of the growth of the various strains of *A. scabies*,

TABLE 10

The influence of sulfur oxidation by Thiobacillus thiooxidans upon the development of A. scabies

TH. THIOOXIDANS	A. SCABIES STRAIN NO.	RELATIVE GROWTH	FINAL REACTION	NH_3 IN 100 GM. SOIL	ACTINOMYCETES PRESENT IN 1 GM. OF SOIL
		Soil itself			
		No sulfur			
			pH	*mgm.*	
+*		0	5.6	7.3	0
+	254	1	5.9	7.5	660,000
+	257	2	6.0	13.6	13,000,000
+	259	2	6.0	9.7	14,500,000
−	259	2	6.0	14.7	17,000,000
		0.1 per cent sulfur			
+		0	2.8	7.1	0
+	254	T	5.8	7.1	18,000
+	257	T	5.8	9.5	0
+	259	T	5.8	7.5	500,000
−	259	1	5.8	10.3	3,000,000
		Soil with 1 per cent dried blood			
		No sulfur			
+		0	5.6	8.7	0
+	254	3	6.7	42.0	22,000,000
−	254	3	7.2	65.8	40,000,000
+	257	3	7.5	79.5	400,000,000
−	257	3	7.5	89.6	100,000,000
+	259	3	7.3	82.0	100,000,000
−	259	3	7.5	82.6	124,000,000
		0.15 per cent sulfur			
+		0	5.6	8.6	0
+	254	T	5.8	12.8	275,000
−	254	1	6.0	23.2	9,000,000
+	257	0	5.6	8.0	0
−	257	3	6.4	32.3	17,000,000
+	259	2	5.8	39.0	5,000,000
−	259	3	6.3	56.0	130,000,000

* + indicates flasks inoculated with sulfur oxidizing organism; −, uninoculated.

as indicated by numbers and ammonia formation. However, the pH values are not those that we would expect from the previous experiments. We would expect that the reaction, in which the limitation of the growth of *A.*

scabies took place, should be at a pH of 5.0 or less. We find flasks, however, in which no growth of *A. scabies* took place, to have a pH value of 5.6 and even 5.8. The only explanation for that phenomenon is the very complexity of the problem and the fact that there was present such a large amount of organic matter. Possibly the sulfuric acid formed from the oxidation of sulfur was present on the surface of the soil particles, sufficient to exert a preventative action upon the germination and growth of *A. scabies*. When the soil is shaken up with water, for the pH determination, the acid may be rapidly neutralized by the buffers present in the soil, in the form of proteins and inorganic colloids. We will be able to answer this complicated problem more definitely when our information of the relation between the acid formation and surface phenomena of the soil as well as the diffusion of the acid will be more extended.

The important conclusion to be drawn from this experiment is the fact that the presence of sufficient sulfur with the sulfur oxidizing bacterium can prevent the development of Actinomyces species that cause potato scab.

INFLUENCE OF SOIL TYPE, MOISTURE, ETC., UPON THE DEVELOPMENT OF ACTINOMYCES SCABIES

According to Wollenweber (38), scab is not abundant in heavy loam and clay soils and humus-rich moor soils. Light sandy soils, especially in dry years are inclined very favorably to scabbiness. Millard (25) called attention to the fact that peat soils are usually free from scab, while in sandy or gravelly soils, especially where these have been liberally supplied with lime, the disease is prevalent. He explains this by the fact that the introduction of fresh organic matter remedies this defect since it serves as a food (acting as a decoy for the fungus), thus the potato escapes the attack.

It would seem to be rather contradictory to observations of those who studied the activities of actinomycetes in the soil. Conn (3), Waksman (31), Krainsky (16) found them to be quite abundant in soils rich in organic matter. Of course, the observation of Millard (25) that the organic matter serves as a sort of decoy for the *A. scabies* is untenable, both from the principles underlying activities of microörganisms and from available data.

The very accurate studies of Gillespie and Hurst (10) on the Caribou and Washburn soil types in Maine would seem to point to just the opposite results from the observations of the other investigators. The Washburn loam which, due to its low situation, favors an accumulation of muck and peat, is much more subjected to infection by *A. scabies*. Gillespie and Hurst pointed out quite accurately that the reasons for this difference are to be looked for in the difference in soil reaction as measured by the hydrogen-ion concentration. No attempt in this direction has been made by either the German (38) or the English (25) investigators.

We must also keep in mind that, in the presence of an abundance of organic matter, there is an abundant development of fungi and acid producing bacteria. These may produce enough acid from the decomposition of the organic matter to limit the growth of *A. scabies*. As a matter of fact, in slightly acid soils, a small amount of scab may be controlled by the use of green manure, which is rapidly decomposed by various soil microörganisms with the formation (temporary probably) of sufficient organic acids to control scab.

As to the influence of moisture and soil type, a brief summary of experiments carried on for that purpose will suffice here.

The optimum condition for the growth of various strains of *A. scabies* is found at a moisture content of about 60 to 65 per cent of the maximum moisture holding capacity of the soil. This optimum is not much different from that commonly employed as the optimum for the activities of soil bacteria and fungi.

When various amounts of sand were added to a medium loamy soil, the best growth of *A. scabies* was obtained with the mixture containing 25 per cent of sand. Upon a further addition of sand, growth rapidly decreased, till, in pure sand, the amount of growth (as indicated by the formation of ammonia from dried blood) was less than one-fourth that in the soil itself.

SUMMARY

1. The limiting acid reaction for the growth of *Actinomyces scabies* in culture solutions, properly buffered, and in soil, varies with the strain. For the majority of strains, the limiting acid reaction is about pH 5.0–5.2; some strains may grow even at pH 4.8, while others will begin to develop only at pH 5.3–5.6. These results bear out, in the main, the results of Gillespie.

2. The saprophytic soil actinomycetes seem to be more acid resistant than *A. scabies* strains.

3. By the use of the proper amount of sulfur inoculated with *Thiobacillus thiooxidans*, an acid reaction is obtained which will control the growth of *A. scabies* in the soil.

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MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: I. THE MATHEMATICAL INTERPRETATION OF NUMBERS OF MICROÖRGANISMS IN THE SOIL[1]

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INTRODUCTORY

In 1909, Fischer (8) published a paper in which he came to the conclusion that we do not possess as yet a proper method for a bacteriological analysis of the soil, and that we have no proper information as yet for the interpretation of the fertility of the soil from a bacteriological point of view. Were we to consider that same question today, we should have to come to the same conclusion. All attempts to interpret crop production from a microbiological point of view have not been rich in consequences and some investigators, who have considered a few of the methods carefully, such as bacterial numbers, ammonification, denitrification, etc., have come to the conclusion that they are practically worthless for the interpretation of soil fertility. The only exception to this statement may be found in some of the recent investigations on nitrification.

This lack of definite information and the unreliability of the information that we do possess is due to a number of factors, chief among which is the lack of standard methods employed. Not only do the methods used at present in soil microbiological research vary in the various laboratories and different importance is attached to them, but samples taken from the soil and treated by the same methods in the same laboratory are often found to give varying results. This may be due to a number of factors: 1. The methods employed in the various laboratories are not standardized. 2. The work is usually carried out with very few unrepresentative samples taken from a small area. 3. The soil is considered as constant and usually no allowance is made for the variations between the different samples. 4. The small amount of exact information that we possess on the microbial flora of the soil and their activities. 5. Very little attempt is usually made to consider the soil micro-flora and its activities as a whole,—ordinarily, only isolated phenomena are considered. This can well be illustrated by the fact that nitrogen metabolism of bacteria is usually considered without a study of the carbon metabolism, as in the case

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of the so-called ammonification studies. 6. Last but not least, the fact that the soil is usually considered in the static condition of equilibrium, so far as soil microörganisms are considered, while it is actually in a dynamic condition, with the equilibrium continually shifted. This can be readily illustrated by referring to the well known Remy method, wherein a small quantity of soil is added to a definite amount of a sterile solution of known composition, then measuring the change that has taken place. This method is good enough for measuring the strength or activity of an unchangeable substance whether of chemical or biological origin (as in the case of enzymes), where the reaction is mono-molecular as far as the substance in question is concerned. In the case of a living soil flora, this method is entirely at fault, since the reaction is bi- tri-, and poly-molecular. It is assumed that the microörganisms do not multiply during the process of the reaction,—actually they not only multiply rapidly but some organisms utilize the products of others, with a host of antagonistic and stimulating substances formed in the process of the reaction, complicating greatly the study of the individual phenomena.

It was, therefore, deemed of greatest importance to take up first a study of the various functions used in the study of soil microbiology and interpret the results not only from a microbiological, but also from a chemical and physico-chemical viewpoint and particularly to investigate the variability of the methods themselves and of the soil as far as the function in question is concerned. The first 3 papers of this series deal with the question of numbers of soil micro-organisms, as determined by the plate method, and their relation to soil fertility.

THEORETICAL

In a bacteriological examination of the soil, the investigator is usually satisfied with a small number of samples taken from a limited area and regards the results obtained from a composite mixture of these samples, as accurate for the whole field. The variation between the samples, however, is of great enough importance to require very careful consideration. If this holds for an examination of the chemical soil constituents and crop production, it applies more so to a bacteriological soil analysis, where the very methods employed involve large errors which are usually not considered. The importance of calculating the mathematical error involved in an investigation has been recognized not only by the astronomer, physicist and chemist, but by the biologist as well. This allows us to judge not only the exactness of our investigation but also, when, on repeating the experment we obtain a different set of data, we could find out whether this change is only within the limits of error of the investigation or involves some new effect, which causes the change in the results.

There are only few reports on record dealing with the calculation of the mathematical error involved in the study of bacteriological activities in the soil, of which the two most important ones are those of Budinov (4) dealing

with bacterial numbers in general and of Waynick (12) dealing with nitrification. Other attempts have been made to get some information on the variability in the actvities of microörganisms, but they either dealt with a function of doubtful importance in interpreting soil phenomena, e.g., ammonification, or no mathematical interpretation was attempted or both, as by Allison and Coleman (1).

Although this paper deals only with a study of numbers of microörganisms, the formulae presented are readily applied also to the variability in the study of other functions. The author felt justified in developing the formulae in this paper in view of the fact that it has not been done in any paper (except that of Budinov (4)) dealing with bacterial numbers and activities and even in the standard reference texts on statistics, such as that of Davenport (6). The need for the presentation of the derivation of formulae used in the mathematical interpretation of results became apparent, when, on examining the various texts, it was found that the statistician and mathematician used different formulae for calculating the average deviation. Davenport, for example, gives

$$\sigma = \sqrt{\frac{\Sigma d^2}{n}}$$

while Mellor (10) uses the formula

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

This may lead to a great deal of confusion, particularly in agricultural literature. Davenport does not state the method of the derivation of the particular formula and the justification for its use. In deriving the formula for the probable error of the mean, Davenport (6), Waynick (12), and others state that this is obtained by dividing the average deviation by the square root of n, which would make

$$Em = \pm 0.6745 \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

which would be the case only when the second formula for the average deviation is used.

The author has been assisted in developing the formulae for the average errors by the standard treatises on the theory of errors and methods of least squares by Bartlett (3), Carvallo (5), Woodward (14), and the more special texts, but particularly by a paper of Budinov (4), which appeared in 1910, in Russian, and which seemed to have escaped the attention of both European and American investigators.

To determine bacterial numbers in the soil, three methods are available: 1, the plate method; 2, the dilution method; and 3, the direct counting method. These methods are discussed in detail in the following paper and reasons are presented for selecting the plate method as the best one, under the present

conditions. The limitations involved in the use of this method as well as details in technic will be fully discussed in that paper, so that we may limit ourselves here only to the method of the mathematical interpretation of the results. In brief, the soil samples are brought into the laboratory and are diluted with sterile tap water to give dilutions of 1:10, 1:1000 and 1:100,000; the first dilution is shaken for just 5 minutes by hand, the last dilution is used for the preparation of plates, in such a manner as to allow 40–200 colonies to develop on a plate. Where the soil is known to run much higher or much lower in numbers, the dilutions are made accordingly. Synthetic agar (egg-albumen) is used as a culture medium. The plates are incubated 7 days at 25°C., unless otherwise stated.

To count bacteria on the plate, three methods are commonly employed: (1) counting with the naked eye all the colonies on the plate, (2) use of counting plate, (3) use of microscope. The first is the most reliable and involves the least error. Of course where a large number of colonies are allowed to develop on the plate, which would necessitate the use of the counting plate or even the microscope there is greater probability of obtaining a greater variety of representative soil forms, and the errors involved in making high dilutions would be to some extent eliminated. The advantage is more than compensated by the fact that there is a great competition for the nutrients on the plate, and with lower dilutions, comparatively fewer bacteria will develop, as will be brought out in the following paper. To get a representative average, with the method of counting all bacteria on the plate, many plates have to be used. This as well as the fact that the counting of all the bacteria on the plate depends also a great deal on the sharpness of vision of the individual observers led various investigators to adopt one of the two other methods.

Since only the method of counting all colonies on the plate has been used in the experiments reported in this paper, we will limit ourselves primarily to a discussion of the errors involved in this method of counting. Some attention will also be given to the use of the counting plate, while the counting by means of the microscope will be omitted from this discussion. The mathematical considerations involved in the three methods of counting are quite similar, with only slight modifications. A mathematical interpretation of experimental results in the growth of plant is found in the papers of Wood (13), Wood and Stratton (14) and Davis (7). The tables given by Davenport (6) and Barlow (2) have been used in calculating the results.

Each set of observations involves occasional and constant errors, positive and negative from the mean. The occasional errors are eliminated as much as possible by the use of improved methods. The error of an observation is the difference between the result of the observation and the true value of the quantity. If the observation is s, the quantity measured q, the error x, then

$$x = s - q$$

The most probable value, which is the closest approximation to the true value

that could be obtained from the series of observations is calculated by means of the method of least squares.

If the same quantity is measured many times and each time we obtain the difference (or residual) between the observed quantities and the mean value, then, by dividing the sum of the differences (residuals) by the number of observations, we obtain the mean arithmetical error. The residual bears the same relation to the true error that the most probable value bears to the true value. With the increase in the number of observations, the most probable value approaches the true value, while residuals approach the corresponding true errors. In this case, the limit of the error is 0, since the positive and negative declinations from the mean are occasional errors, not counting, of course, the constant errors.

If n plates are used for making a quantitative bacteriological count of a given soil, a is the number of colonies developing on the plates, z is the true value, while z_n is the most probable value obtained from counting n plates, or the arithmetical mean value, then we have

$$z_n = \frac{\Sigma a}{n}$$

In other words, the mean (z_n) is found by dividing the sum of all the determinations by the number of determinations. Since the true value is z, each determination varies from the true value by

$$x_1 = z - a_1;\ x_2 = z - a_2;\ x_3 = z - a_3 \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot\ x_n = z - a_n$$

If $$\frac{\Sigma x}{n} = \Delta z$$

then $$z = \frac{\Sigma a}{n} + \frac{\Sigma x}{n} = z_n + \Delta z$$

Δz is the error of the mean from the true value of the given quantity. In view of the fact that the error can be either positive or negative, the error of the mean squared can then be expressed as the square of the sum of the errors divided by their number.

$$(\Delta z)^2 = \left(\frac{\pm x_1 \pm x_2 \pm x_3 \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \pm xn}{n}\right)^2$$

By expanding and combining this equation, we find

$$(\Delta z)^2 = \frac{\Sigma x^2}{n^2}$$

as n increases indefinitely. The average error of a single observation from the arithmetical mean or standard deviation (σ) is usually assumed to be equal to $\sqrt{\frac{\Sigma x^2}{n}}$, in that case $\Delta z = \frac{\sigma}{\sqrt{n}}$. The standard deviation is a measure of the reliability of any one result; the chances are even that any one determination taken

at random will differ from the average by this deviation. In other words, the error of the mean (Δz) is inversely proportional to the square root of the number of observations; the error of the mean of 100 observations will be 10 times less than the average error of single observations. The laws of chance cannot be expected to give normal results with small numbers, therefore, the larger the number of observations, the more reliable is the average obtained. This holds true, in the following investigations, both for the number of plates used in counting microörganisms and for the number of soil samples taken from one particular plot.

Since we know only the arithmetical mean (z_n) and not the true value (z), we have

$$z = z_n + \Delta z, \text{ then}$$
$$x_1 = z_n + \Delta z - a_1 = (z_n - a_1) + \Delta z$$
$$x_2 = z_n + \Delta z - a_2 = (z_n - a_2) + \Delta z$$
$$\cdots\cdots\cdots\cdots\cdots\cdots$$
$$\cdots\cdots\cdots\cdots\cdots\cdots$$
$$x_n = z_n + \Delta z - a_n = (z_n - a_n) + \Delta z$$

By squaring and adding we find

$$\Sigma x^2 = \Sigma (z_n - a)^2 + n \Delta z^2$$

Dividing the above equation by n,

$$\frac{\Sigma x^2}{n} = \frac{\Sigma (z_n - a)^2}{n} + \Delta z^2$$

Substituting σ^2 for the first part of the equation and $\frac{\sigma}{\sqrt{n}}$ for Δz, we find that

$$\sigma^2 = \frac{\Sigma (z_n - a)^2}{n} + \frac{\sigma 2}{n}, \text{ or}$$

$$\sigma = \sqrt{\frac{\Sigma (z_n - a)^2}{n - 1}}, \text{ therefore}$$

$$\Delta z = \sqrt{\frac{\Sigma (z_n - a)^2}{n (n - 1)}}$$

The average deviation is thus found to be different from that originally assumed. The probable error of the individual observation or arithmetical mean is obtained by multiplying the above formulae by $\pm$ 0.6745:

$$Em = \pm 0.6745 \sqrt{\frac{\Sigma (z_n - a)^2}{n (n - 1)}}$$

Since for a single observation, $\sqrt{n} = 1$, the probable error is

$$\Sigma s = \pm 0.6745 \times \sigma$$

Approximately two-thirds of the determinations are expected to lie on both sides of the mean, within that deviation.

The probable error of the standard deviation is obtained from the formula $\pm 0.6745 \frac{\sigma}{\sqrt{2n}}$. The coefficient of variability (C.V.) according to Davenport (6) is the percentage ratio of the standard deviation (σ) from the mean. The probable error of the coefficient of variability $= \pm 0.6745 \frac{\text{C.V.}}{\sqrt{2n}}$. By the use of formulae for the calculations of the errors of observation, we can now figure out the error of the mean arithmetical value of the plate counts. The formula used for the standard deviation in the following investigations, is the second derived formula, namely

$$\sigma = \sqrt{\frac{\Sigma (z_n - a)^2}{n - 1}} \tag{1}$$

However, for comparison with the work based on Davenport's formula, a formula is given whereby the results obtained from the derived formula (1) can easily be transformed into results that would be obtained by using the Davenport formula (2); m (in other words σ, according to Davenport) $= \sigma \sqrt{\frac{n-1}{n}}$,

$$m(\sigma) = \sqrt{\frac{\Sigma (z_n - a)}{n}} \tag{2}$$

If N is the total number of colonies, a the number on each plate, and u the amount of dilution, then

$$N = au$$

In the mathematical interpretation of the results obtained by any soil bacteriological function, we must consider two sets of variables: that of the soil and that of the method. Both of these are of equal importance: the first has been well illustrated in the nitrification studies of Waynick (12) and the second in the mathematical analysis of the quantitative determination of bacteria in milk by Budinov (4). Waynick (12) dealt with the question of nitrification, and the method commonly employed gives rather constant results, although something will have to be said about the actual true value of the method as interpreting a biological function. In the study of such a function as the determination of bacterial numbers in the soil, the method itself, as pointed out above, is quite a variable factor,—it should therefore be considered first, before the variability of the soil itself, as far as numbers of microörganisms are concerned is considered.

VARIABILITY OF THE PLATE METHOD FOR COUNTING SOIL MICROÖRGANISMS

For the study of the variability of the method, a sample of soil was taken from a quantity of soil kept under optimum moisture conditions for the last

eight months. A dilution of 100,000 was made and 62 plates were poured. A few plates were also prepared from a dilution of 1,000 of the same soil. The plates made from the first dilution were used for the variability of the

TABLE 1

The use of the counting plate in determining the numbers of microörganisms in the soil, by the plate method

COLONIES	$(x_n - a)$	$(x_n - a)^2$	COLONIES	$(x_n - a)$	$(x_n - a)^2$
51	+17.4	302.76	31	− 2.6	6.76
56	+22.4	501.76	17	−16.6	275.56
48	+14.4	207.36	20	−13.6	184.96
59	+25.4	645.16	19	−14.6	213.16
52	+18.4	338.56	19	−14.6	213.16
41	+ 7.4	54.76	22	−11.6	134.56
39	+ 5.4	29.16	24	− 9.6	92.16
34	+ 0.4	0.16	36	+ 2.4	5.76
36	+ 2.4	5.76	44	+10.4	108.16
29	− 4.6	21.16	57	+23.4	547.56
33	− 0.6	0.36	41	+ 7.4	54.76
24	− 9.6	92.16	49	+15.4	237.16
25	− 8.6	73.96	42	+ 8.4	70.56
14	−19.6	384.16	38	+ 4.4	19.36
23	−10.6	112.36	26	− 7.6	57.76
18	−15.6	243.36	29	− 4.6	21.16
19	−14.6	213.16	28	− 5.6	31.36
23	−10.6	112.36	24	− 9.6	92.16
35	+ 1.4	1.96	32	− 1.6	2.56
43	+ 9.4	88.36	34	+ 0.4	0.16
19	−14.6	213.16	23	−10.6	112.36
28	− 5.6	31.36	22	−11.6	134.56
52	+18.4	338.56	16	−17.6	309.76
55	+21.4	457.96	31	− 2.6	6.76
36	+ 2.4	5.76	24	− 9.6	92.16
49	+15.4	237.16	27	− 6.6	43.56
47	+13.4	179.56			
51	+17.4	302.76	2016*	− 5.0	8357.40*
33	− 0.6	0.36			
36	− 2.6	6.76	Mean = 33.6 ± 1.03		
34	+ 0.4	0.16	σ = ±11.9 ±0.74		
34	+ 0.4	0.16	*C. V.* = 35.4 ± 2.18 per cent		
26	− 7.6	57.76	Δz = ±1.53		
39	+ 5.4	29.16	*Em* = 3.06 per cent		

* Totals of both columns.

method by counting all colonies, while the plates obtained with the low dilution were counted utilizing the counting plate.

In this study all the colonies, besides the fungi, were counted without any attempt made to differentiate between the bacteria and actinomycetes. Table 1 contains the results obtained from the use of the counting plate. The

microörganisms were so numerous, that they were counted at the end of 3 days.

When the plates are counted by means of the counting plate, we must keep in mind that we have to trust the firm preparing the plate, in its accuracy. The method of calculating the error applies to this method in a way similar to the one worked out for counting the whole plate, only here we count various sections of the plate.

The first column represents the number of colonies, the second, $(z_n\text{-}a)$, gives the errors obtained by subtracting the individual counts from the arithmetical mean, the third column, $(z_n\text{-}a)^2$, contains the squares of the errors.

The average of the 60 counts on the plate is 33.6. The sum of the squares is found to be 8,357.40, therefore the average deviation is, for each count:

$$\sigma = \sqrt{\frac{\Sigma\,(z_n - a)^2}{n-1}} = \sqrt{\frac{8{,}357.40}{59}} = \pm 11.9$$

$$C.V. = \frac{11.9 \times 100}{33.6} = 35.4 \text{ per cent}$$

The error of the mean observed value is

$$\Delta z = \frac{\sigma}{\sqrt{n}} = \pm 1.53$$

The most probable error is then equal to

$$Em = \pm 1.53 \times 0.6745 = \pm 1.03 = 3.06 \text{ per cent}$$

Since the number of the sections on the plate was 60 and the dilution 1000, the total number of microörganisms, exclusive of fungi, obtained by this method is $1000 \cdot 60 \cdot (33.6 \pm 1.03) = 2{,}016{,}000 \pm 61{,}800$ per gram of moist soil.

It is interesting to note that the sample gave in the same period of incubation, an average of 561 colonies with 10,000 dilution and 64 colonies with 100,000 dilution.

The second experiment deals with the variability involved in the use of a large number of plates, with a dilution commonly employed in determining the numbers of microörganisms in the soil, whereby only between 40 to 200 colonies are allowed per plate, and a long enough period of incubation is employed to allow a maximum development of microörganisms. Tables 2 and 3 contain the counts made on the same soil used in the previous experiment, with a dilution of 100,000. The plates were incubated for 7 days at 25–27°C.

In counting the colonies developing on the plates, first all colonies, except fungi, were counted, then the actinomycetes were determined separately.

The arithmetical mean of all the 62 plates, considering at first the bacteria (in other words, bacteria+actinomycetes) is 73.68.

$$\sigma = \sqrt{\frac{8458.5488}{61}} = \pm 11.78$$

$$C.V. = \frac{11.78 \times 100}{73.68} = 15.99 \text{ per cent}$$

$$\Delta z = \frac{\sigma}{\sqrt{n}} = \frac{11.78}{\sqrt{62}} = \pm 1.50$$

$$Em = \pm 1.50 \times 0.6745 = \pm 1.012 = 1.36 \text{ per cent}$$

The total number of microörganisms, exclusive of fungi, is then found to be $(73.68 \pm 1.012) \times 100{,}000 = 7{,}368{,}000 \pm 101{,}200$, without considering the moisture of the particular soil.

When the coefficient of variation and the most probable error of the two methods of counting bacteria are compared, we find that it is less than a half, by the use of a large number of plates and counting all colonies, than in the case of counting the colonies on the Petri dish by means of a counting plate. For this reason and other reasons given above, the method of making high dilutions, using a large number of plates and counting all the colonies on the plate is to be preferred.

When the actinomyces colonies are considered, we find the arithmetical mean to be 24.85.

$$\sigma = \sqrt{\frac{1149.6950}{61}} = \pm 4.34$$

$$C.V. = \frac{4.34 \times 100}{24.85} = 17.47 \text{ per cent}$$

$$\Delta z = \frac{4.34}{\sqrt{62}} = \pm 0.55$$

$$Em = \Delta 0.55 \times 0.6745 = \pm 0.371 = 1.50 \text{ per cent}$$

The *C. V.* and most probable error are found to be for actinomycetes somewhat higher than that for the number of bacteria plus actinomycetes. A possible explanation for that will be suggested below. Attention may be called here to the work of Johnstone (9) on the probable error involved in a bacteriological analysis of the fecal pollution of shell fish. The frequency curve obtained was highly asymmetrical. A dilution of 250 cc. was prepared and 20 plates were counted. The highest frequency ran with the lowest counts. Since the calculation of the probable value of a measure presupposes a symmetrical distribution (Gaussian), it is not legitimate to apply the probable error to a highly asymmetrical distribution. Michael (10) suggests that in that case some influence other than "chance" is operative, and a function should be substituted whose distribution is Gaussion, which will enable the separation. The function suggested is the logarithmic value. The most prob-

able number of the bacteria will then be not the arithmetical mean, but the geometric mean. This may be true enough, but another error is overlooked, namely the use of too high a dilution, which allows too few bacteria to develop

TABLE 2

The variability of numbers of bacteria in the soil as determined by the common plate method

COLONIES	$(z_n - a)$	$(z_n - a)^2$	COLONIES	$(z_n - a)$	$(z_n - a)^2$
69	− 4.68	21.9024	60	−13.68	187.1424
74	+0.32	0.1024	62	−11.68	136.4224
71	− 2.68	7.1824	99	+25.32	641.1024
69	− 4.68	21.9024	94	+20.32	412.9024
64	− 9.68	93.7024	81	+ 7.32	53.5824
71	− 2.68	7.1824	86	+12.32	151.7824
67	− 6.68	44.6224	75	+ 1.32	1.7424
56	−17.68	312.5824	85	+11.32	128.1424
59	−14.68	215.5024	67	− 6.68	44.6224
68	− 5.68	32.2624	74	+ 0.32	0.1024
73	− 0.68	0.4624	97	+23.32	543.8224
87	+13.32	177.4224	88	+14.32	205.0624
70	− 3.68	13.5424	83	+ 9.32	86.8624
75	+ 1.32	1.7424	60	−13.68	187.1424
77	+ 3.32	11.0224	71	− 2.68	7.1824
68	− 5.68	32.2624	72	− 1.68	2.8224
76	+ 2.32	5.3824	66	− 7.68	58.9824
84	+10.32	106.5024	74	+ 0.32	0.1024
76	+ 2.32	5.3824	68	− 5.68	32.2624
106	+32.32	1044.5824	81	+ 7.32	53.5824
74	+ 0.32	0.1024	67	− 6.68	44.6224
81	+ 7.32	53.5824	89	+15.32	234.7024
76	+ 2.32	5.3824	69	− 4.68	21.9024
98	+24.32	591.4624	87	+13.32	177.4224
70	− 3.68	13.5424	78	+ 4.32	18.6624
52	−21.68	469.0224	62	−11.68	136.4224
63	−10.68	114.0624	55	−18.68	348.9424
59	−14.68	215.5024			
72	− 1.68	2.8224	4568*	+ 0.84	8458.5488*
73	− 0.68	0.4624			
51	−22.68	514.3824	Mean = 73.68 ±1.01		
74	+ 0.32	0.1024	σ = 11.78 ±0.72		
56	−17.68	312.5824	*C. V.* =15.99 ± 0.97 per cent		
83	+ 9.32	86.8624	Δz = ± 1.50		
76	+ 2.32	5.3824	*Em* = 1.36 per cent		

* Totals of both columns.

on the plate. It will be pointed out in the following paper (as shown also by other investigators) that the number of bacteria allowed per plate should be between 40 and 200. Where larger numbers of colonies develop, there is apt to be overcrowding; with fewer numbers, the samples are apt to be unrepre-

sentative. Out of the 20 plates used by Johnstone (9), 19 contained less than 30 colonies per plate; this accounts for the fact that an asymmetrical curve has been obtained and accounts for the influence other than "chance" mentioned by Dr. Michael (11).

TABLE 3

The variability of numbers of actinomycetes in the soil as determined by the common plate method

COLONIES	$(x_n - a)$	$(x_n - a)^2$	COLONIES	$(x_n - a)$	$(x_n - a)^2$
21	−3.85	14.8225	18	− 6.85	46.9225
23	−1.85	3.4225	24	− 0.85	0.7225
29	+4.15	17.2225	27	+ 2.15	4.6225
28	+3.15	9.9225	27	+ 2.15	4.6225
20	−4.85	23.5225	23	− 1.85	3.4225
23	−1.85	3.4225	32	+ 7.15	51.1225
18	−6.85	46.9225	28	+ 3.15	9.9225
25	+0.15	0.0225	33	+ 8.15	66.4225
26	+1.15	1.3225	24	− 0.85	0.7225
19	−5.85	34.2225	18	− 6.85	46.9225
25	+0.15	0.0225	28	+ 3.15	9.9225
24	−0.85	0.7225	27	+ 2.15	4.6225
23	−1.85	3.4225	22	− 2.85	8.1225
24	−0.85	0.7225	32	+ 7.15	51.1225
28	+3.15	9.9225	23	− 1.85	3.4225
26	+1.15	1.3225	26	+ 1.15	1.3225
30	+5.15	26.5225	26	+ 1.15	1.3225
21	−3.85	14.8225	25	+ 0.15	0.0225
20	−4.85	23.5225	31	+ 6.15	37.8225
28	+3.15	9.9225	24	− 0.85	0.7225
23	−1.85	3.4225	34	+ 9.15	83.7225
22	−2.85	8.1225	29	+ 4.15	17.2225
25	+0.15	0.0225	38	+13.15	172.9225
28	+3.15	9.9225	31	+ 6.15	37.8225
23	−1.85	3.4225	26	+ 1.15	1.3225
18	−6.85	46.9225	28	+ 3.15	9.9225
20	−4.85	23.5225	25	+ 0.15	0.0225
22	−2.85	8.1225			
23	−1.85	3.4225	1541*	+ 0.30	1149.6950*
19	−5.85	34.2225			
24	−0.85	0.7225	Mean = 24.85 ± 0.37		
18	−6.85	46.9225	σ = 4.34 ± 0.27		
23	−1.85	3.4225	*C. V.* = 17.47 ± 1.14		
24	−0.85	0.7225	Δs = ±0.55		
19	−5.85	34.2225	*Em* = 1.50 per cent		

* Totals of both columns.

VARIABILITY OF THE SOIL IN RELATION TO NUMBERS OF MICROÖRGANISMS

To study the variability of the soil itself as far as bacterial numbers are concerned, a field plot was selected. The plot is one of the experimental plots at the College Farm at New Brunswick, used by the Soil Department

of this station for the study of utilization of nitrogenous fertilizers. The particular plot received annually for the last 14 years, 320 pounds $NaNO_3$ per acre, in addition to minerals. An outline of the plots and the rotation of crops used will be found in the following paper. In 1921, the plot was in timothy. Samples were taken 2 to 3 weeks after the crop had been harvested.

TABLE 4

Bacterial colonies in the upper 6 inches of soil of Plot 9A, dilution: 1–100,000, and moisture content of samples

NUMBER OF SAMPLE	COLONIES	MOISTURE CONTENT	NUMBER OF SAMPLE	COLONIES	MOISTURE CONTENT
		per cent			*per cent*
1	97.0 ± 2.65	12.3	30	186.8 ± 6.27	12.5
2	99.2 ± 3.34	12.1	31	128.3 ± 5.74	14.5
3	86.3 ± 4.50	12.3	32	148.8 ± 3.71	13.6
4	161.0 ± 6.88	13.2	33	116.7 ± 5.53	13.7
5	132.2 ± 7.35	14.3	34	112.8 ± 5.50	13.4
6	119.4 ± 5.84	14.3	35	138.5 ± 4.40	11.8
7	102.5 ± 4.39	13.7	36	146.6 ± 4.01	12.9
8	121.2 ± 5.96	13.5	37	181.8 ± 1.58	12.6
9	111.8 ± 7.52	12.9	38	151.5 ± 3.25	12.6
10	133.7 ± 3.13	13.2	39	175.4 ± 5.26	13.0
11	146.2 ± 2.17	13.2	40	132.7 ± 3.79	12.4
12	118.0 ± 5.34	13.6	41	99.3 ± 2.01	12.8
13	135.0 ± 6.42	13.5	42	86.5 ± 3.64	10.9
14	136.8 ± 5.15	12.2	43	95.7 ± 3.08	11.1
15	164.7 ± 3.75	13.6	44	89.7 ± 4.71	11.1
16	120.0 ± 3.86	12.5	45	107.7 ± 2.27	10.7
17	132.2 ± 3.44	13.2	46	113.7 ± 6.79	12.2
18	177.8 ± 7.34	14.8	47	109.3 ± 3.93	11.8
19	189.8 ± 6.76	12.9	48	96.8 ± 4.68	13.0
20	111.5 ± 4.81	13.2	49	89.5 ± 2.96	13.2
21	125.5 ± 6.49	12.1	50	164.6 ± 2.63	11.3
22	101.3 ± 3.29	11.6	51	112.5 ± 7.44	12.8
23	226.8 ± 11.82	13.3			
24	167.6 ± 8.11	13.1	Mean=128.9 ± 2.97		12.8 ± 0.09
25	111.2 ± 5.14	11.6	σ=31.5 ± 2.1		0.9 ± 0.10
26	100.3 ± 7.74	11.4	*C. V.*=24.44 ± 1.63 per cent		7.66
27	92.8 ± 3.12	12.8	Δz=±4.4		±0.14
28	118.2 ± 3.60	14.4	*Em*=2.30 per cent		0.7
29	147.7 ± 2.06	14.3			

Several rather heavy rainfalls preceded the sampling of the plot by about a week.

The plot is $\frac{1}{10}$ of an acre in size, 66 x 33.3 feet. The samples were taken by means of a 1-inch sampling tube to a depth of $6\frac{1}{2}$ inches, 3–4 feet from the edge, and about 5 x 8 feet apart, 6 rows in width and 8 rows in length. The soils were plated out soon after they were brought into the laboratory, six plates were used for each sample. A larger number of plates should have

been used, but, in that case, not all the samples could have been plated out in one day; it was thought that the error involved in plating out the different samples on the different days would be greater than the use of a smaller number of plates. Due to lack of space, only the arithmetical mean for each sample is reported in the columns. The results and the moisture content of the samples are given in table 4. The extremes in numbers of microörganisms (bacteria and actinomycetes) are found to be 86.3 ± 4.50 and 226.8 ± 11.82 colonies, which represent 8,630,000 ± 450,000 and 22,680,000 ± 1,182,000 per gram of moist soil. Were the results for bacterial numbers in all the other nitrogen plots reported here (see following paper), they would be found to come, with one or two exceptions, within this range. In other words, *wherever determinations of bacterial numbers in the soil are based only on one sample, the results are worthless, due to the great variability of the soil in respect to numbers of microörganisms.*

There was in some cases an evident correlation between the position of the sample and the number of microörganisms. The samples taken at the edges of the plot, particularly in the corners, gave lower numbers than the samples coming from the middle of the plot. This may be due, in some cases at least, to an uneven distribution of the fertilizer. Perhaps it may be due, in some cases, to the lower moisture content. This would lead us to conclude that the determination of numbers of microörganisms in the soil should be based not on one but on a number of samples taken from various parts of the particular field.

The combined variability of the method and the soil

With these considerations in mind, we can now proceed to the development of a practical method for determining the number of microörganisms in the soil which would allow both for the variability of the plate method and that of the soil, without involving too great an excess of time and energy and which could be used in routine work. For the following experiment, a check plot was used, which had received no fertilizer application at all for the last 14 years.

Samples were taken from five different parts of the plot, each sample representing a mixture of three borings taken 1–2 feet apart. The samples were thoroughly mixed, placed in sterile jars, and plated out as soon as brought into the laboratory. Ten plates were used for each sample, so that fifty plates were poured for the five samples taken from the same plot. A dilution of 100,000 was used in all cases. The plates were incubated for 7 days at 25°C., then the microörganisms were counted. The results are tabulated in table 5, the clear spaces differentiating the different samples.

The average of all 50 plates obtained from the five samples of the same soil is $\frac{4173}{50} = 83.46 \pm 1.5$ for the total microörganisms, exclusive of the fungi; $\frac{556}{50} = 11.3 \pm 0.3$ for the actinomycetes; and $\frac{138}{50} = 2.76 \pm 0.16$ for the fungi.

The probable error for the number of microörganisms, exclusive of fungi, is 1.8 per cent. Since the dilution is 100,000 and the moisture content of

TABLE 5

Numbers of microörganisms in the soil, as shown by the plate method

BACTERIA AND ACTINOMYCETES		ACTINOMYCETES		FUNGI	
Colonies		*Colonies*		*Colonies*	
94	79	9	7	6	3
84	83	8	13	3	6
73	103	11	14	2	3
61	98	10	12	2	5
99	76	9	10	2	2
83	76	15	12	3	3
67	112	9	13	3	4
77	101	15	15	3	2
65	84	7	12	6	3
91	56	11	11	2	2
64	106	13	14	2	4
96	101	10	16	0	3
81	89	11	13	2	3
53	84	8	12	4	5
96	116	7	14	4	1
69	76	8	12	4	1
72	82	15	11	7	1
62	93	13	9	1	1
69	76	12	15	7	0
77	84	7	9	3	1
106	52	11	4	2	2
93	78	9	17	4	1
98	61	9	11	2	1
87	93	12	13	0	1
91	106	6	21	4	2
Total = 4173		Total = 565		Total = 138	
Mean=83.46 ± 1.5		Mean=11.3 ± 0.30		Mean= 2.76 ± 0.16	
σ=15.69 ± 1.07		σ= 3.16 ± 0.20		σ= 1.73 ± 0.11	
C. V.=18.8 ± 1.27 per cent		*C. V.*=27.96±1.89 per cent		*C. V.*=62.67±4.23 per cent	
Δz=±2.22		Δz=±0.45		Δz=±0.245	
Em=1.8 per cent		*Em*=2.7 per cent		*Em*=5.8 per cent	

the particular soil is 10 per cent, the total number of microörganisms, exclusive of fungi, in the particular plot, on the basis of dry soil, is found by multiplying the colonies by 100,000 and by $\frac{10}{9}$:

$$(83.46 \pm 1.5) \times 100{,}000 \times \frac{10}{9} = 9{,}273{,}333 \pm 166{,}667$$ (true within 1.8 per cent).

In the case of actinomycetes, the probable error of the mean is 2.7 per cent. By multiplying by 100,000 and $\frac{10}{9}$, we obtain the most probable number of

actinomycetes, within the limits of the methods used for their determination, as 1,255,556 ± 33,333 per gram of dry soil.

The error of the determination of actinomycetes is thus found to be once and a half as great as that for the determination of total number of microorganisms exclusive of the fungi. This can be readily explained by the fact that there are greater errors involved in counting actinomycetes colonies than in counting the total number of colonies: in the last case all colonies are counted, the fungi being distinguished very readily, and, given a long enough period of incubation and little chance for overcrowding, particularly by spreaders and fungi, the colonies can be quite accurately counted; in the case of actinomycetes, we add the error of mistaking sometimes an actinomyces for a bacterial colony and *vice versa*. Of course, the fact that the number of actinomycetes is less than the total number of colonies will tend to increase the probable error.

The most probable number of fungus colonies is found to be

$$2.76 \pm 0.16$$

The most probable value of the total number of fungi in the particular soil is then:

$(2.76 \pm 0.16) \times 100{,}000 \times \frac{10}{9} = 306{,}667 \pm 17.778$ per gram of dry soil true within 5.8 per cent.

There is a tremendous jump in the probable error of determining fungi by this particular method. While the most probable error is true for the total number of microörganisms, exclusive of fungi, within 1.8 per cent and for the actinomycetes within 2.7 per cent, it is true for the fungi only within 5.8 per cent, a range more than three times as great as for the other soil microörganisms. This cannot be explained, as was done in the case of the actinomycetes, by the errors made in recognizing the colonies, since the fungus colonies are well recognized, particularly when the plates are incubated 7 days.

The reason for this large error lies elsewhere, namely in the inefficiency of the plate method for the determination of numbers and activities of fungi in the soil. Since the fungi exist in the soil both in the form of spores and in the form of aerial mycelium, and because of the great abundance of spores formed in a single head of an *Aspergillus*, *Penicillium* and *Mucor*, the error involved is greatly increased: while one small clump of soil may be free from sporulating mycelium, another clump very near to that may contain even one sporulating piece of mycelium which will account for the error. As a matter of fact all we need to do for a lucid explanation of this phenomenon is to point out the upper and lower groups of colonies of fungi in the column in the table.

But another important factor is to be considered, that of the small number of fungi developing on the plates primarily designed, both as to medium and dilution, for the counting of bacteria and actinomycetes.

This factor is sufficient to omit entirely the counting of fungi on the common plate used for the counting of bacteria and actinomycetes. However, it has been used in the experiments submitted in the following paper, due to the fact that some information was obtained even with such an error, which is sufficient to condemn it, for the particular study made. But this is only a specific instance, while as a general method of procedure it is not to be recommended. If it is designed to obtain more or less accurate information on the fungus numbers in the soil, the method proposed below should be used.

To turn back to the table, we find that interesting observations can be made on comparing the number of colonies obtained from each soil sample. For this we must separate the five groups, of ten plates each, which represent the five composite samples taken from the same field. We will limit ourselves in this instance, only to the total number of colonies, exclusive of the fungi, since this is the least variable factor and, therefore, the most reliable. On averaging the five groups, we obtain the following number of colonies for each sample: 79.4, 73.9, 91.4, 92.5, 80.1. The total average is 83.46.

The most probable errors for these 5 groups are:

$\pm$ 2.77, $\pm$ 2.99, $\pm$ 2.16, $\pm$ 3.92, $\pm$ 3.31, respectively equivalent to

3.50 per cent, 4.05 per cent, 2.36 per cent, 4.25 per cent, 4.11 per cent

The error is found to be much greater than when it is based upon a count of 50 plates representing all the five samples. When we calculate the probable error of these five samples, we find it to be equivalent to $\pm$2.45 or 2.93 per cent. By limiting ourselves to fewer and fewer samples we get a larger and larger error; by taking fewer and fewer plates for each sample we also increase our error. By increasing the number of samples taken and the number of plates used for each sample, we appreciably decrease the probable error.

The reason for the indefiniteness of the results obtained from counting the number of microörganisms, and lack of correlation between these results and actual crop production is to be looked for in the fact that the great majority of determinations were based upon one or two samples, in many instances not even composite samples, and 2–4 plates were used for making the determinations. Among other errors involved we must point to the lack of proper culture media (not standard in composition), in some cases a short incubation period, whereby not all the colonies were allowed to develop, and errors in the technic. These questions will be taken up in detail in the following paper.

As an example of the error usually obtained in a quantitative bacteriological soil analysis, we may point to the excellent work of Hiltner and Störmer who stated to have obtained an error, in the determination of numbers of bacteria in the soil, of 8–10 per cent and in some cases 15 per cent. Only one sample was taken (representing however a thorough mixture of several samples) and 4 plates were used. It is interesting to note that these investigators, as long as 20 years ago, recognized the importance of the large error involved,

but they tried to correct it by improving the technic. The use of a larger number of plates for the reduction of the error has been recognized, but not carried out.

The question whether a quantitative determination of the number of microorganisms can be taken as a function of soil bacteriology will be answered only in the following paper. However, on the basis of the mathematical interpretation of the results, we might state here that the determination of the total number of microörganisms based on a number of soil samples with a number of plates used for each sample and proper technic, whereby the probable error is reduced to less than 2 per cent, seems to offer an appropriate biological function.

THE DETERMINATION OF NUMBERS OF FUNGI IN THE SOIL

The mathematical study of the distribution of fungi in the soil reported in the last table coupled with other considerations considered by the writer elsewhere, would lead us to condemn the plate method for counting fungi as a method for the study of the activities or even distribution of these organisms in the soil. However, another method could be suggested here, namely the

TABLE 6

The numbers of fungi in the soil

COLONIES	$(z_n - a)$	$(z_n - a)^2$	COLONIES	$(z_n - a)$	$(z_n - a)^2$
36	+ 4.8	23.04	29	− 2.2	4.84
27	− 4.2	17.64	37	+ 5.8	33.64
38	+ 7.8	60.84	29	− 2.2	4.84
30	− 1.2	1.44	26	− 5.2	27.04
24	− 7.2	51.84	44	+12.8	163.84
26	− 5.2	27.04	32	+ 0.8	0.64
27	− 4.2	17.64	36	+ 4.8	23.04
21	−10.2	104.04	42	+10.8	116.64
26	− 5.2	27.04	35	+ 3.8	14.44
24	− 7.2	51.84	28	− 3.2	10.24
35	+ 3.8	14.44	36	+ 4.8	23.04
37	+ 5.8	33.64	32	+ 0.8	0.64
20	−11.2	125.44	28	− 3.2	10.24
28	− 3.2	10.24	32	+ 0.8	0.64
37	+ 5.8	33.64	33	+ 1.8	3.24
41	+ 9.8	96.04	29	− 2.2	4.84
34	+ 2.8	7.84			
32	+ 0.8	0.64	1248*	+ 1.0	1343.00*
39	+ 7.8	60.84			
31	− 0.2	0.04	Mean = 31.2 ± 0.63		
23	− 8.2	67.24	σ = ± 5.87 ± 0.45		
25	− 6.2	38.44	*C. V.* = 18.8 ± 1.42 per cent		
26	− 5.2	27.04	Δz = 0.93		
33	+ 1.8	3.24	*Em* = 2.0 per cent		

* Totals of both columns.

use of media which will allow only a development of fungi, but not of other microörganisms, so that the final dilution could be made low enough, whereby 25 to 100 colonies of fungi could develop on each plate. The period of incubation should be between 2 and 3 days, at 25°C. Longer periods will favor overgrowth, while a shorter period will not allow all fungi to develop. A medium like raisin agar, which is very acid in reaction or a synthetic medium having a reaction of pH = 3.5–4.0 will be found appropriate, while the dilution may be 0.01 of that used for the plating out of bacteria. Under these conditions, the probable error for the determination of numbers of fungi (spores and pieces of mycelium) is appreciably reduced.

The following table will give som eresults obtained by this method. The same soil used in the last experiment has been used for this study. A dilution of 1000, an incubation period of 3 days at 25–27°C. and raisin agar were employed. The results are self explanatory and need not be discussed any further. The marked reduction in the probable error shows that fungi can be determined quantitatively in the soil only when a special acid medium has been used so that the bacteria and actinomycetes are eliminated, and only the fungi develop on the plate. A low enough dilution should be used to allow 20 to 100 colonies per plate.

SIGNIFICANCE OF A QUANTITATIVE BACTERIOLOGICAL ANALYSIS OF SOIL

Since it is often impossible to report all the details connected with the determinations of numbers of microörganisms, as far as all individual plates for every sample are concerned, it is suggested to designate, by a definite figure, the "weight" or importance to be attached to the results, in addition, of course, to the probable error. The weight to be attached to each sample should be the logarithm of the number of plates used in determining the numbers of microörganisms in a particular sample. The weight to be attached to the numbers of soil samples taken from a particular field or plot should be in direct proportion to that number. Each soil sample should be a composite of two or three samples, if possible. We will then have the following series:

NUMBER OF PLATES	"WEIGHT"			
	1 sample	2 samples	3 samples	10 samples
1	0	0	0	0
2	0.30	0.60	0.90	3.0
3	0.48	0.96	1.44	4.8
4	0.60	1.20	1.80	6.0
5	0.70	1.40	2.10	7.0
6	0.78	1.56	2.24	7.8
7	0.85	1.70	2.55	8.5
8	0.90	1.80	2.70	9.0
9	0.95	1.90	2.85	9.5
10	1.00	2.00	3.00	10.0

A weight less than 1.0 is practically worthless, a weight of 1.0 to 2.0 is fair, 2–3 is good, 3–5 is very good. A higher weight than 5.0, particularly if the samples are composite may involve an excessive amount of work, but will, of course give still more reliable results.

SUMMARY

1. The variability of the common method employed for quantitative determination of microörganisms in the soil is too high, when only 2–3 plates are used, and too much reliability cannot be attached to the results.

2. Only by the use of a large number of plates and by making an accurate determination of the probable error involved can we state definitely how much weight we may attach to the results obtained from a quantitative examination of the microörganisms in the soil.

3. The dilution commonly employed for counting bacteria and actinomycetes is too high for the counting of fungi, so that the results obtained from counting fungi on the same plate with the bacteria and actinomycetes are worthless for most soils. Special acid media, whereby only fungi will develop combined with a low dilution (about 100 of that used for counting bacteria) will give more reliable results.

4. The variability of the soil samples taken from the field is too high, we should be able to attach great importance to determinations based upon a single sample.

5. A number of samples should be taken from each field; these are then composited into fewer samples, which should be used for the bacteriological studies. Even then, we should determine the probable error of our function from a large number of individual samples.

6. By combining a relatively large number of plates with a large number of soil samples, we can work out the probable error involved in a quantitative determination of the microörganisms in the soil.

7. A set of figures for the designation of the "weight" to be attached to the determinations based on the number of plates and soil samples has been suggested.

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